Mechanical modeling & numerical simulation of organogenesis at the shoot apical meristem of *Arabidopsis Thaliana*

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The Morphogenetics Project

Genes

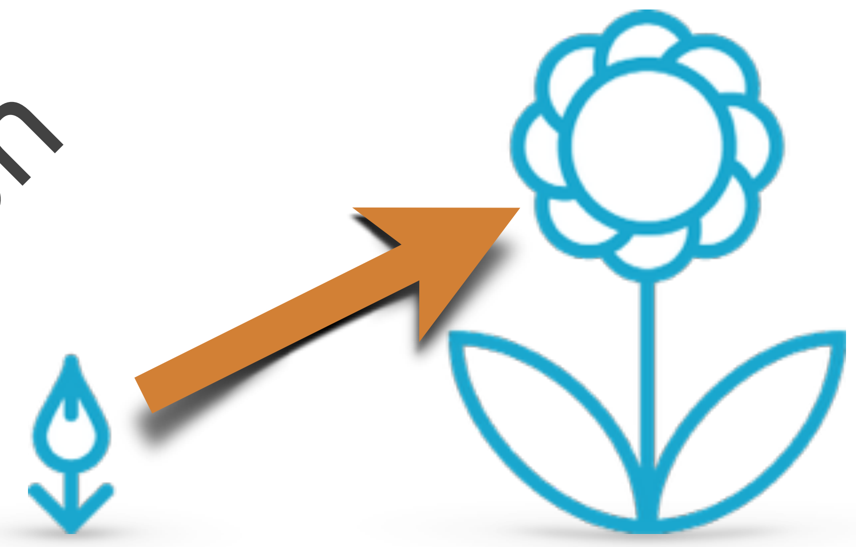
$\{g_1, g_2, g_3, \ldots, g_n\}$

expression

?

regulation

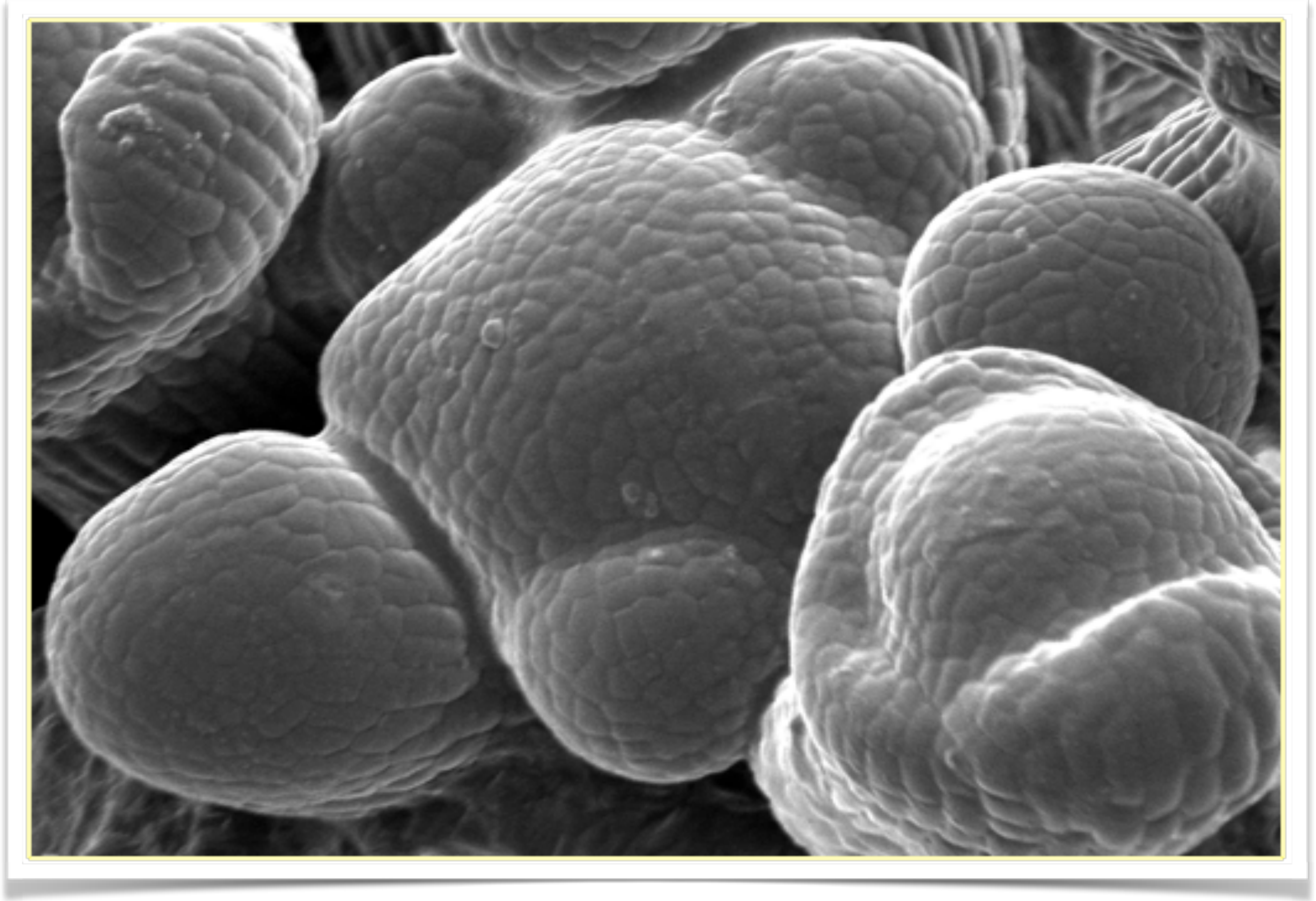
Shape evolution



Tissue = Physical object ⟹ **Shape evolution** is **constrained by** laws of **Mechanics**

Genes must regulate mechanical characteristics of the tissue

- How do genes influence mechanical characteristics ?
- How do mechanical characteristics dictate shape ?



Building up the model

What Biology tells us about the foundations of growth in plants ?

1. In plants, growth is highly dependent on **turgor pressure**.
2. Plant cells feature a **rigid exoskeleton** preventing them to burst

➡**Cell expansion** results from the **yielding of cell wall** under turgor-induced constrains

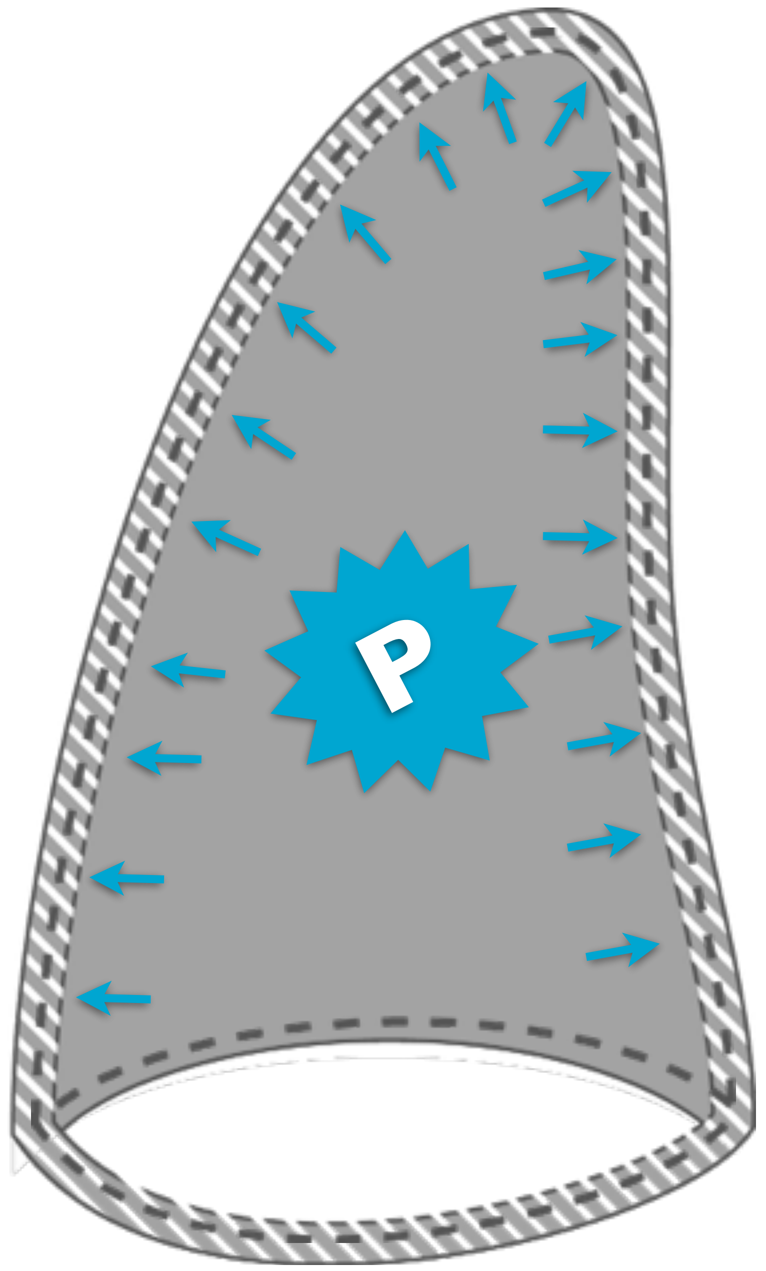
3. **Alteration of the mechanical characteristics** of the tissue appears as the **triggering event**, downstream of Auxin signaling.

Courtesy of Massimiliano Sassi - RDP

Growth = biological process regulated by mechanics

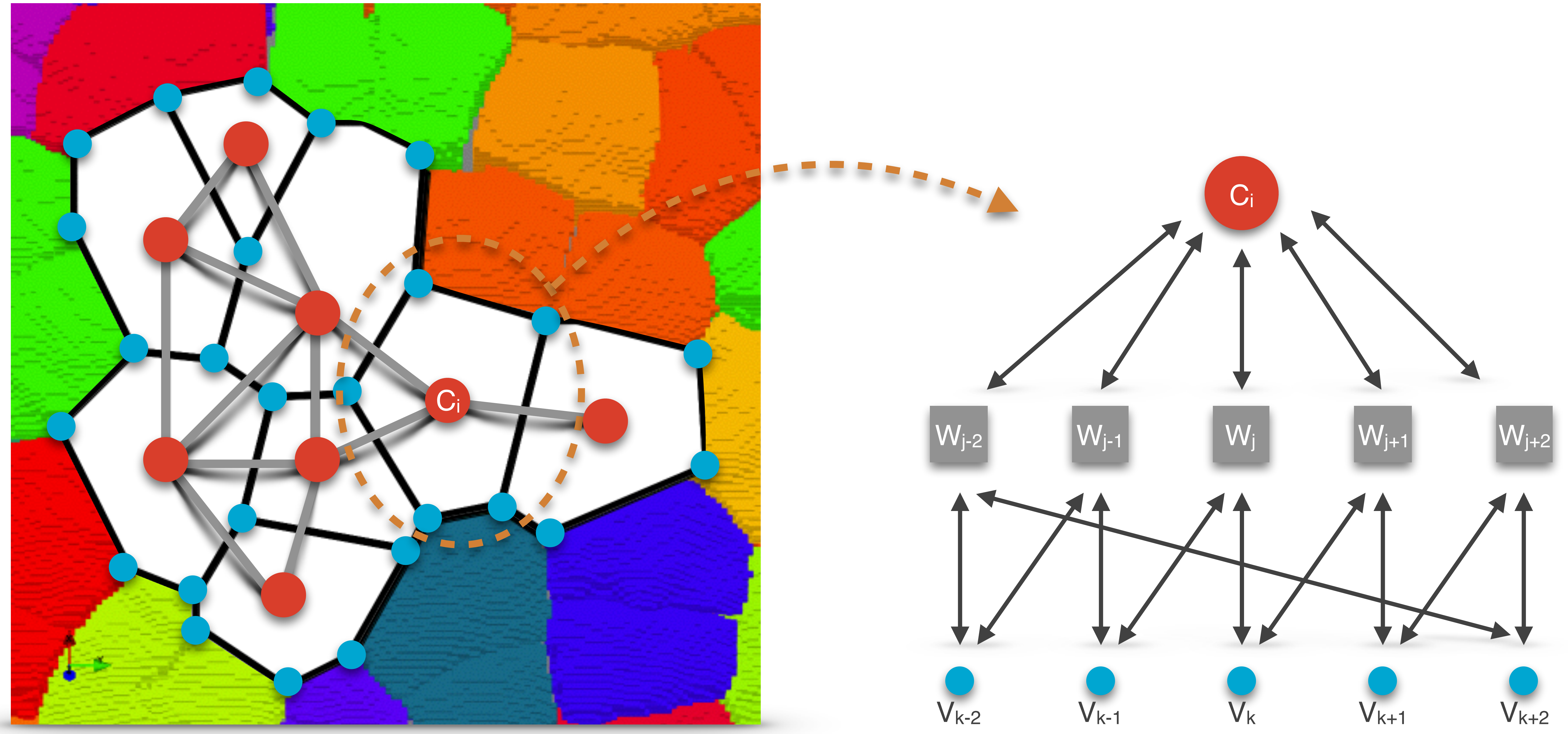
Cell Wall = physical system we are studying (with mechanical characteristics)

Turgor Pressure = constrain (driving force)



The OpenAlea “Tissue Structure”

The tissue is represented as a network of interconnected objects featuring various properties



Courtesy of J. Legrand, RDP

Objects : *Properties*

Cells : *Volume*, *Type*, *Prot. X, Y …*

Walls : *Area*, *Rigidity*, *Transporters…*

Vertices : *Position*

Edges : *Length*, *Orientation*

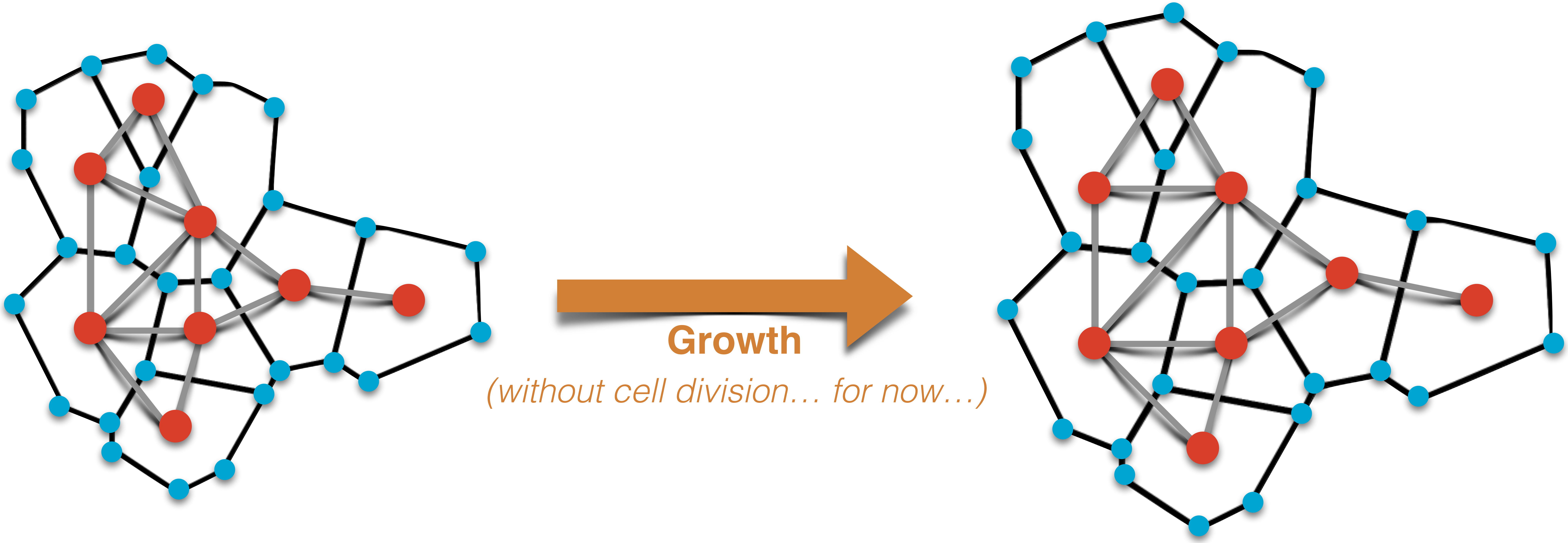
(not represented in 2D)

Multi-scale representation with embedded information:

- Topological: Adjacency graph
- Geometrical: Volumes of cells, surface Area of walls, orientations …
- Mechanical: Rigidity, extensibility …
- Biological: Localization of proteins, TF, TM transporters …

Studying development *in silico*

A biophysical model is required to compute the development of the numerical tissue



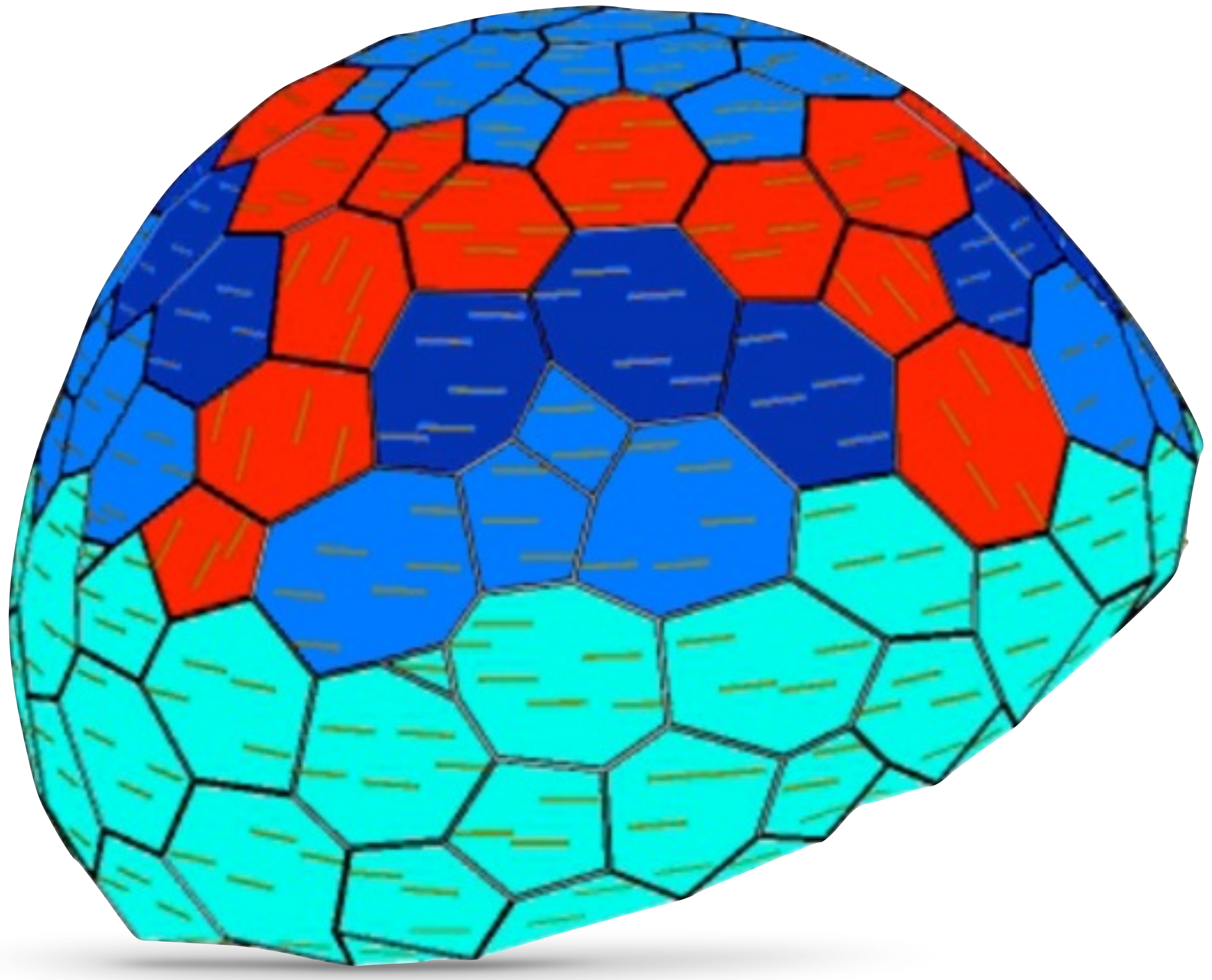
Structure at t_0　　　　*Structure at $t_0+\Delta t$*

To make the numerical tissue structure evolves with time, one has to express cell's expansion rule in a quantitative manner as a function of biological, geometrical & mechanical parameters.

Describing the meristem with continuum mechanics

Outline of today's talk

Courtesy of Massimiliano Sassi - RDP



Macroscopic description of the tissue based on continuum mechanics of deformable solids

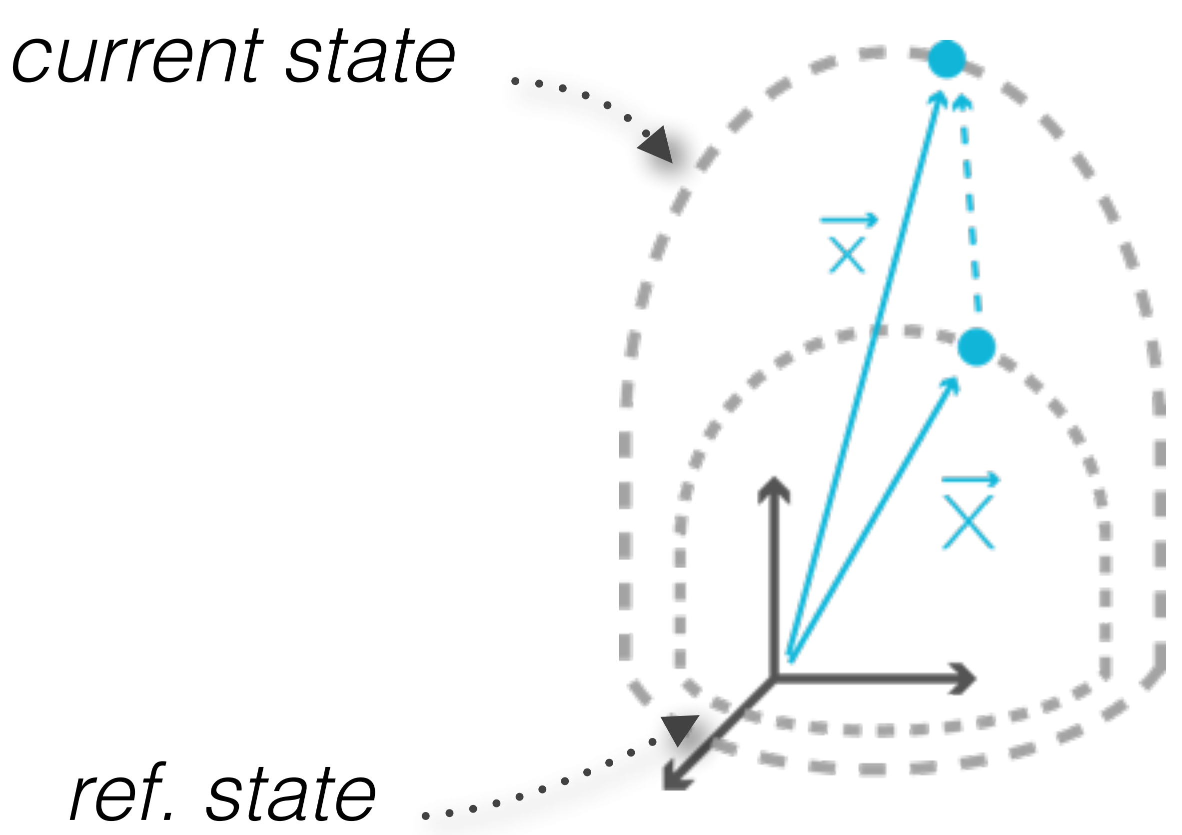
➟ 3 questions:

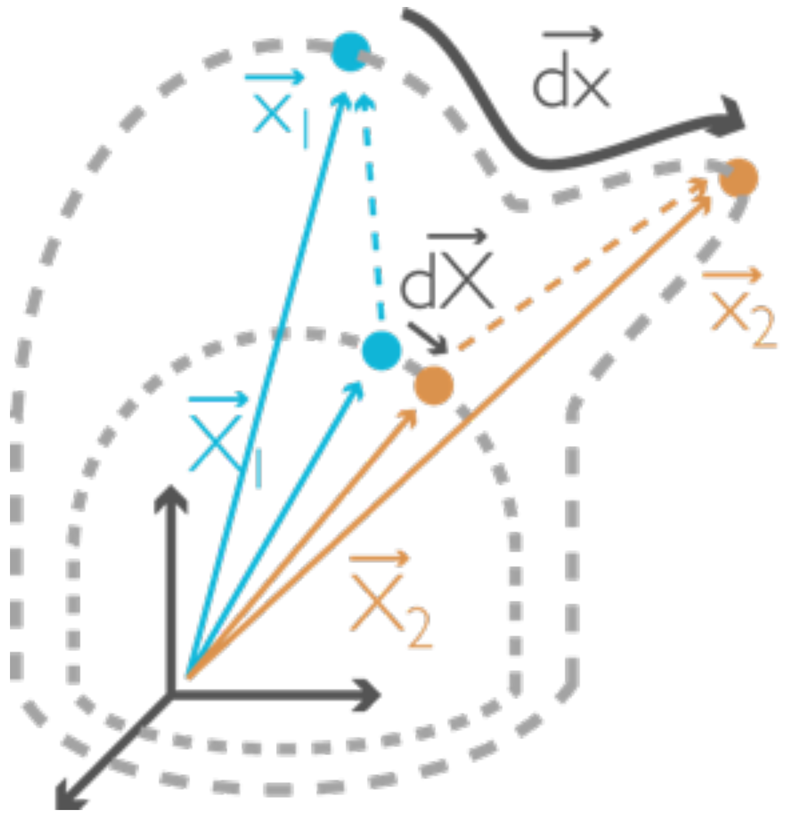
- Geometrical description of the tissue
- Mechanical behavior/properties
- Growth behavior

Geometrical description of the structure

Geometrical description of the tissue

*Parametrization of a shape evolution by the deformation gradient **F***





$$\vec{x}(t) = \vec{\chi}\left(\vec{X}, t\right)$$

$$d\vec{x}(t) = \nabla_X\left[\vec{\chi}\right] \cdot d\vec{X} = F \cdot d\vec{X}$$

χ = displacement

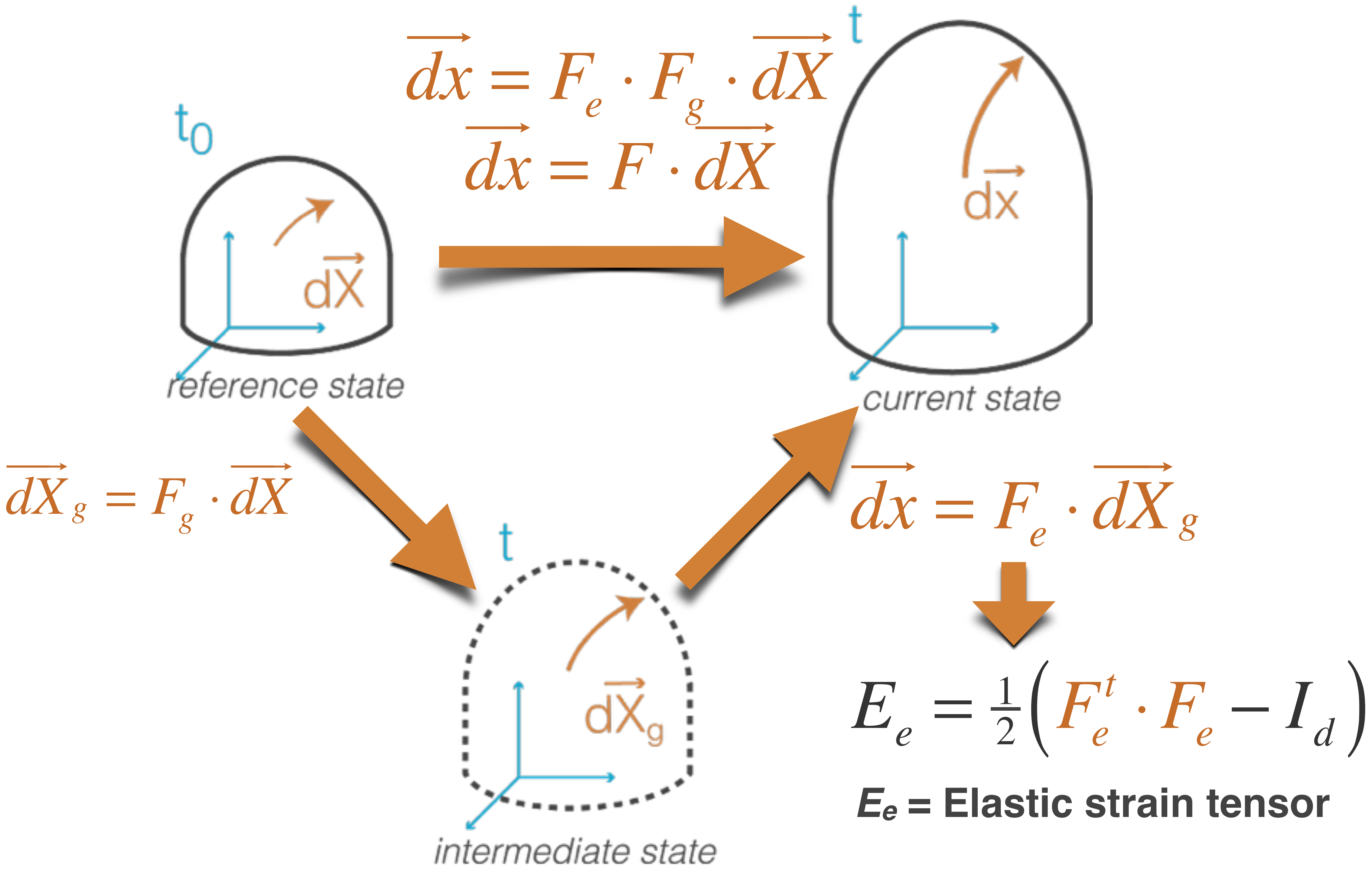
(vector field)

F = deformation gradient

(matrix field)

Geometrical description of the tissue

Reversible V.S. irreversible deformation



$$\overrightarrow{dx} = F_e \cdot F_g \cdot \overrightarrow{dX}$$

$$\overrightarrow{dx} = F \cdot \overrightarrow{dX}$$

$$\overrightarrow{dX}_g = F_g \cdot \overrightarrow{dX}$$

$$\overrightarrow{dx} = F_e \cdot \overrightarrow{dX}_g$$

$$E_e = \tfrac{1}{2}\left(F_e^t \cdot F_e - I_d\right)$$

***E*e = Elastic strain tensor**

multiplicative decomposition

$$F = F_e \cdot F_g$$

Reversible part:
"elastic" behavior
of the cell wall
Mechanics

Irreversible part:
"viscoplastic"
behavior of C.W.
Biology

Mechanics is always faster than Biology

$$\tau_e \ll \tau_g$$

We focus on time such as $t \sim \tau_g \Rightarrow \tau_e \sim 0$

Csq: **Always at mechanical equilibrium**

***E*e** = f(mechanical load)

Mechanical description of the elastic equilibrium

Mechanical description of the cell wall

Elastic properties of the cell wall

Hooke's law of linear elasticity:

$$S_e(P) = \mathbf{H} : E_e$$

$\mathbf{H}$ = mechanical description of the tissue as an elastic linear continuum

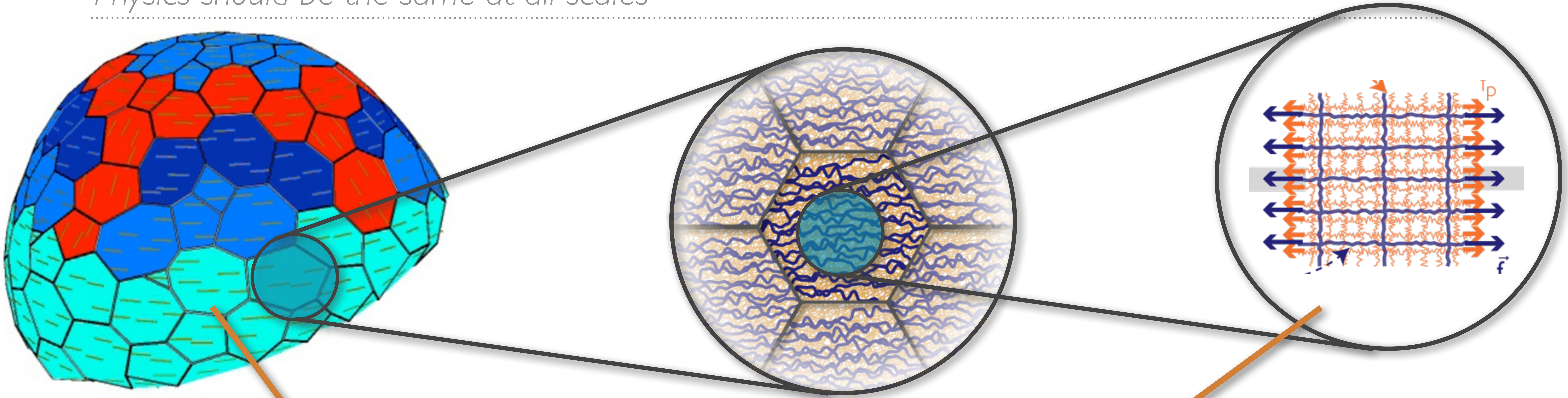
$$\mathbf{H} = \begin{bmatrix} Y_{xeff} & \mu_{xy}Y_{xeff} & \mu_{xz}Y_{xeff} & 0 & 0 & 0 \\ \mu_{yx}Y_{yeff} & Y_{y} & \mu_{yz}Y_{yeff} & 0 & 0 & 0 \\ \mu_{zx}Y_{zeff} & \mu_{zy}Y_{zeff} & Y_{z} & 0 & 0 & 0 \\ 0 & 0 & 0 & G_{xy} & 0 & 0 \\ 0 & 0 & 0 & 0 & G_{yz} & 0 \\ 0 & 0 & 0 & 0 & 0 & G_{zx} \end{bmatrix}$$

How can we relate those coefficients to molecular characteristics of the cell wall ?

With: $Y_{ieff} \; \dfrac{Y_i}{1-\left(\mu_{yx}\mu_{xy}+\mu_{yz}\mu_{zy}+\mu_{zx}\mu_{xz}+\mu_{xy}\mu_{yz}\mu_{zx}+\mu_{xz}\mu_{zy}\mu_{yx}\right)}$

Macro- & microscopic description of the cell wall

Physics should be the same at all scales



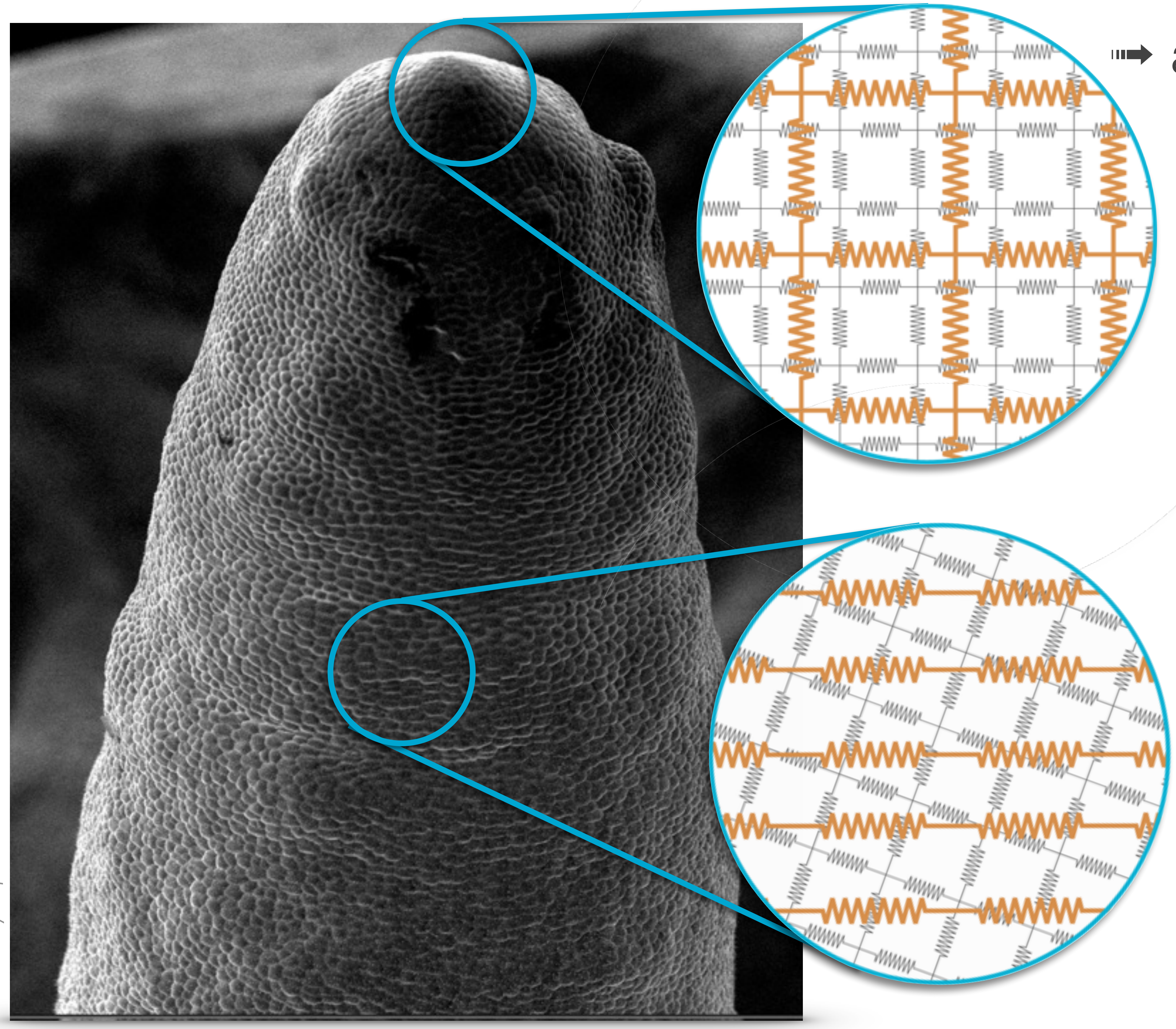
Continuum mechanics
description:
Relation between Stress (**S_e**) & Strain (**E_e**)

Molecular
description:
relation between forces ($\vec{f}$) & stretching (Δl)

Those 2 descriptions have to be the same !

Setting the mechanical characteristics

Elastic properties of the cell wall



Courtesy of Massimiliano Sassi - RDP

➟ at the mesoscopic scale:

An 2D **in-homogenous**, potentially **transverse anisotropic**, elastic linear continuum.

$$\mathbf{H} = \begin{bmatrix} \frac{Y_x}{1-\mu_{xy}\mu_{yx}} & \frac{\mu_{xy}Y_x}{1-\mu_{xy}\mu_{yx}} & 0 \\ \frac{\mu_{yx}Y_y}{1-\mu_{xy}\mu_{yx}} & \frac{Y_y}{1-\mu_{xy}\mu_{yx}} & 0 \\ 0 & 0 & G_{xy} \end{bmatrix}$$

➟ at the microscopic scale:

2 main components:

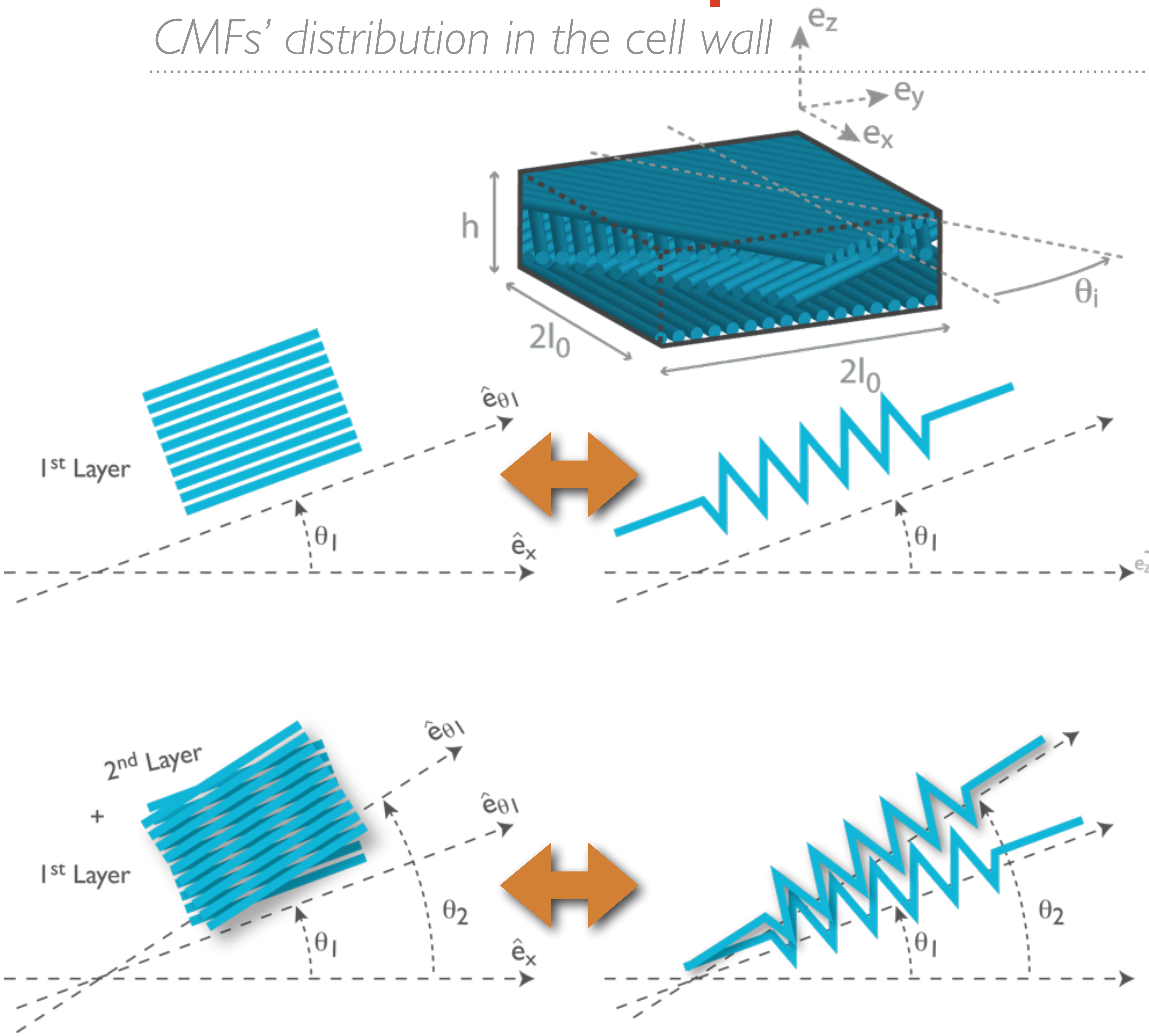
- **pectin** (dense isotropic matrix)
- **cellulose** (big strong fibers)

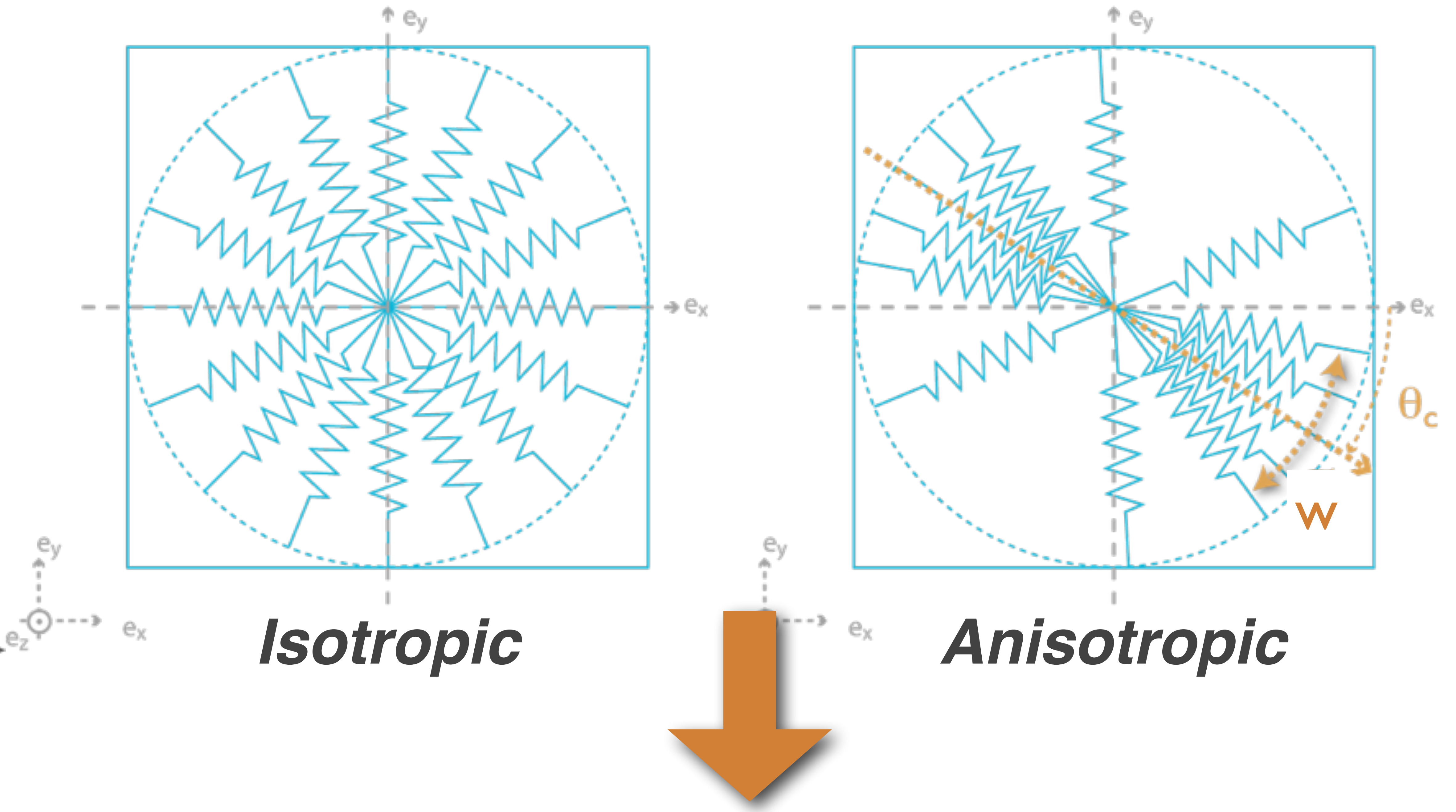
Mechanical anisotropy comes from the orientation of CMFs within the call

➟ Can we express **Y**, **G** & **μ** as function of this orientation ?

Structural description of the cell wall

CMFs' distribution in the cell wall





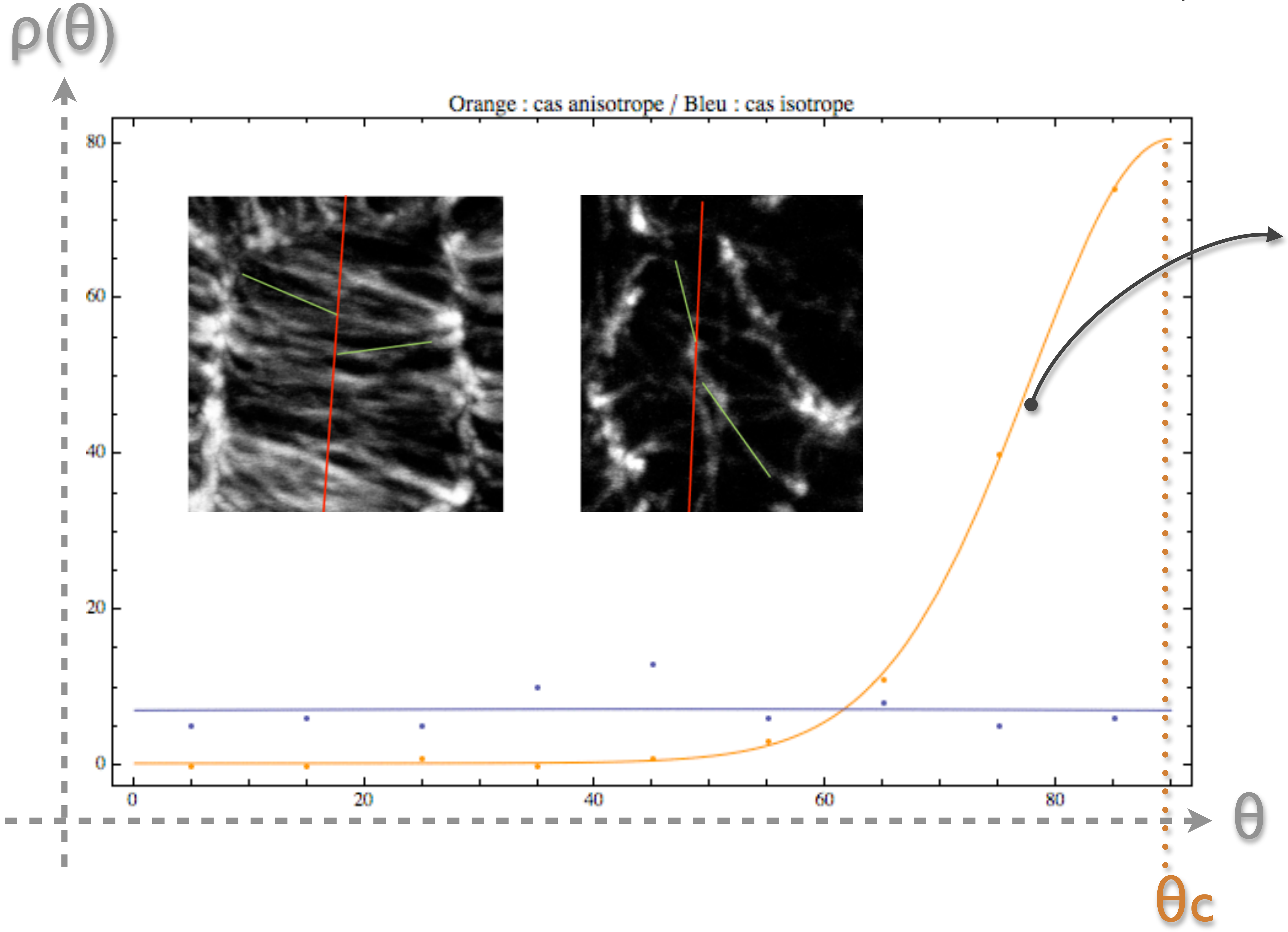
One important parameter:
The **angular density of CMFs**

$$\rho(\theta)=\frac{N(\theta)}{N_{tot}}$$

Why ρ is an important variable ?

Because we have an experimental proxy to estimate it

The Cortical MicroTubules (CMTs) distribution

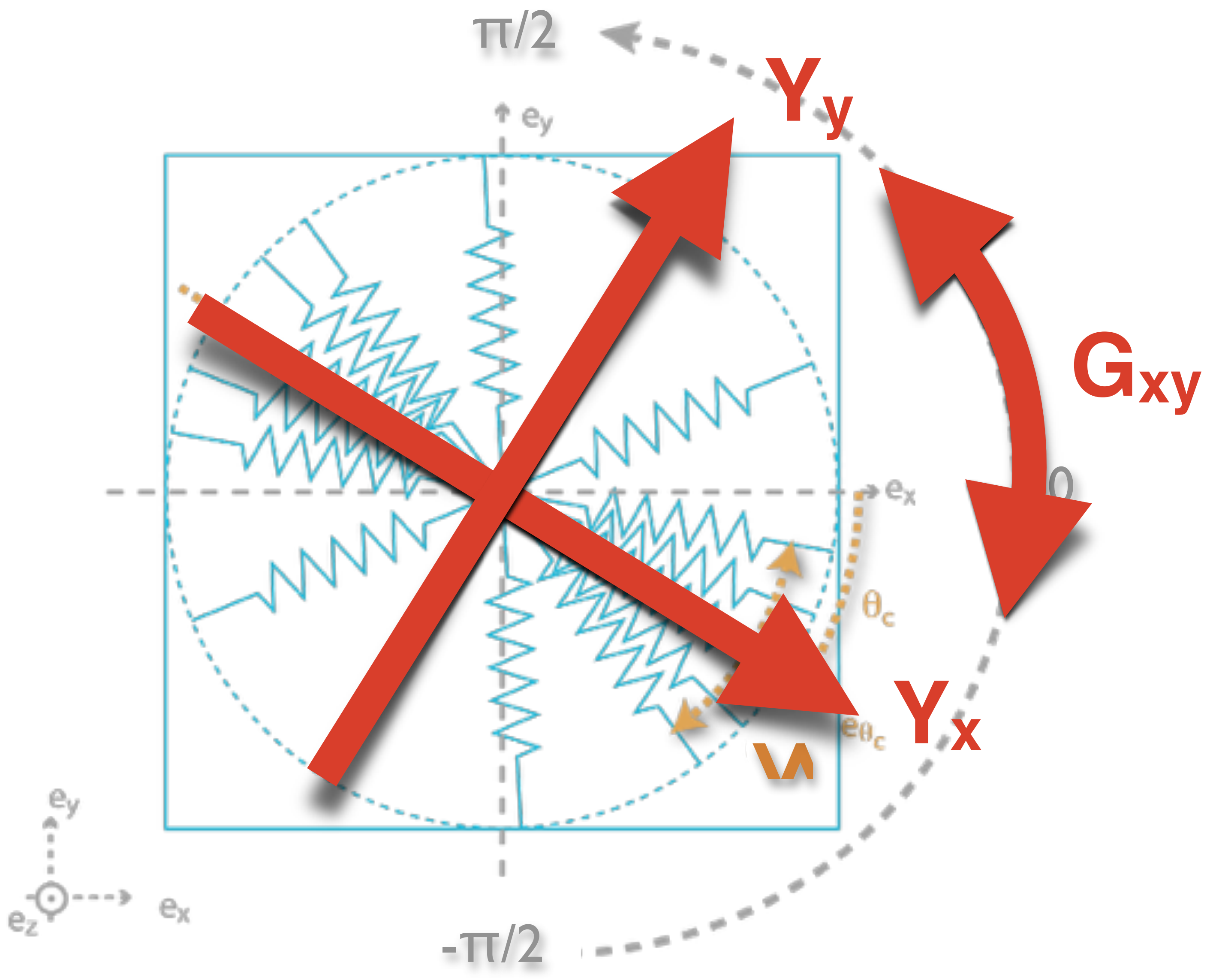


$$\rho(\theta) = \rho_0 \left(\frac{1-\delta}{\pi} + \frac{\delta}{A} Exp\left[-\left(\frac{Sin(\theta - \theta_c)}{w} \right)^2 \right] \right)$$

ρ(θ) is the main molecular characteristic we are interested in.

The "SCARY" slide...

Bridging the gap between macroscopic and microscopic mechanical description of the cell wall



$$\mathbf{H} = \begin{bmatrix} Y_{xeff} & \mu_{xy} Y_{xeff} & 0 \\ \mu_{xy} Y_{xeff} & Y_{yeff} & 0 \\ 0 & 0 & G_{xy} \end{bmatrix}$$

$$Y_{xeff} = \frac{Y_x}{1 - \mu_{xy}\mu_{yx}}$$

$$Y_{yeff} = \frac{Y_y}{1 - \mu_{xy}\mu_{yx}}$$

$$Y_{xeff} = Y_0 \cdot \int_0^{\pi} d\theta \cdot \tilde{\rho}(\theta) \cdot \cos^4(\theta)$$

$$Y_{yeff} = Y_0 \cdot \int_0^{\pi} d\theta \cdot \tilde{\rho}(\theta) \cdot \sin^3(\theta)$$

$$G_{xy} = \frac{Y_0}{4} \cdot \int_0^{\pi} d\theta \tilde{\rho}(\theta) \cdot \sin^2(2\theta)$$

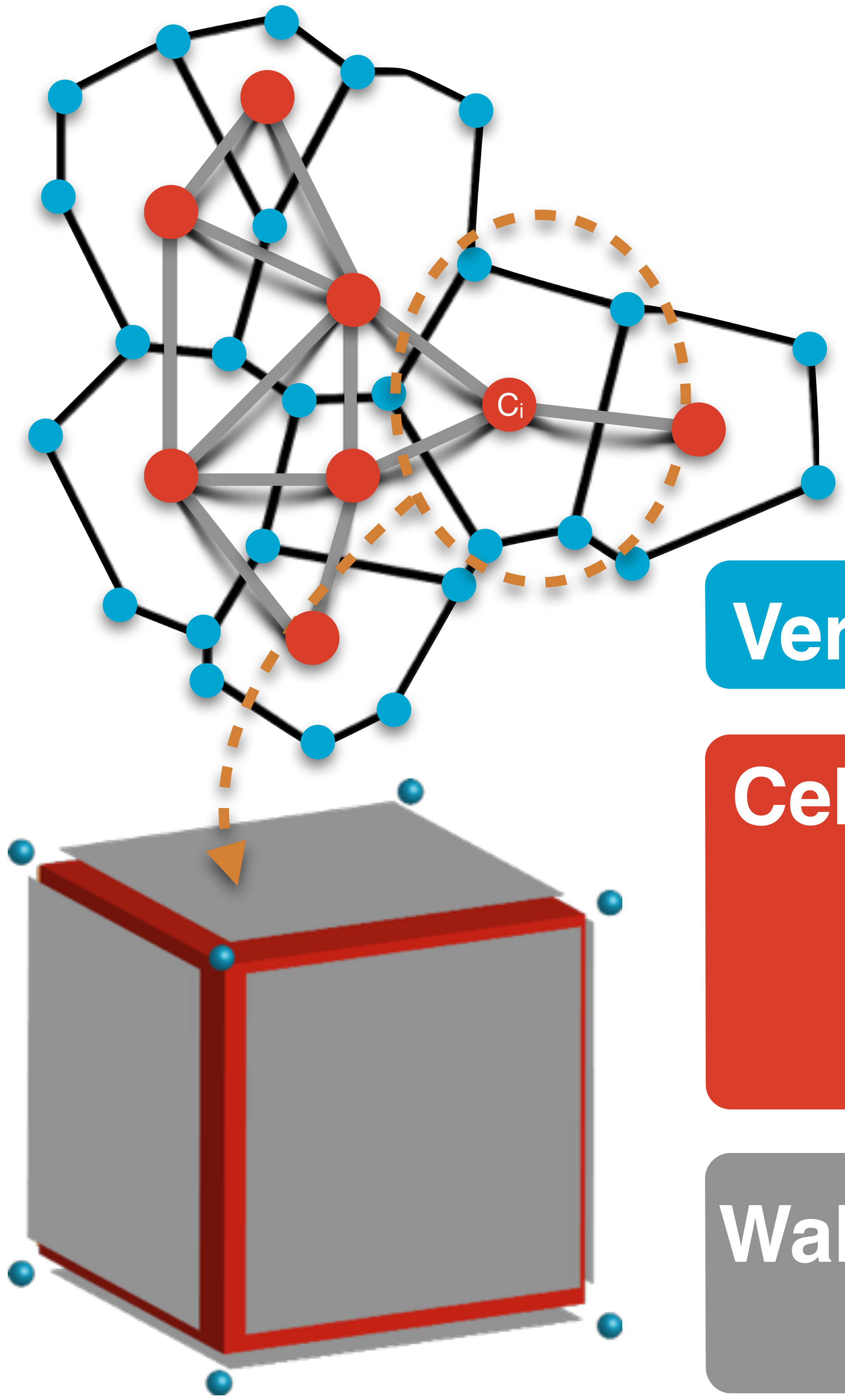
$$\mu_{yx} = \frac{1}{4} \frac{\int_0^{\pi} d\theta \tilde{\rho}(\theta) \cdot \sin^2(2\theta)}{\int_0^{\pi} d\theta \tilde{\rho}(\theta) \cdot \sin^4(\theta)}$$

$$\mu_{xy} = \frac{1}{4} \frac{\int_0^{\pi} d\theta \tilde{\rho}(\theta) \cdot \sin^2(2\theta)}{\int_0^{\pi} d\theta \tilde{\rho}(\theta) \cdot \cos^4(\theta)}$$

With: $Y_0 = k_0 l_0^2 \rho_0$

Intermediate sum up

From CMTs' orientation we can estimate rigidity patterns within the walls



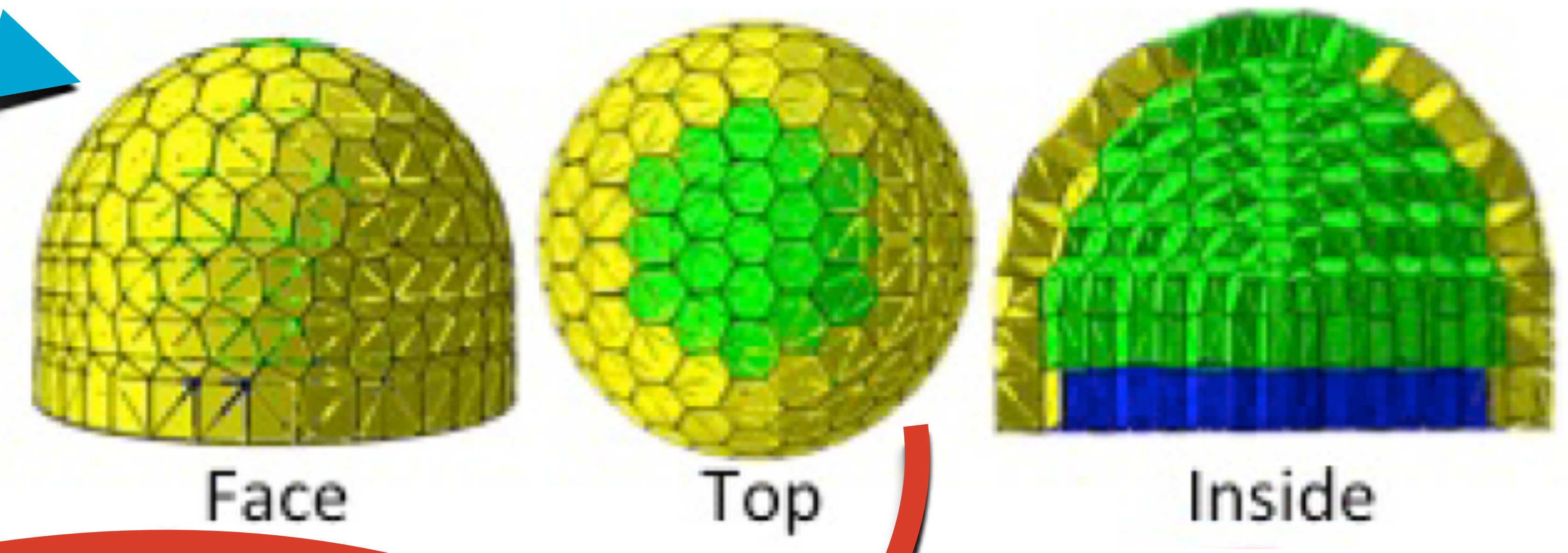
Vertices *Position*

Cells *Turgor Pressure* *CMTs' orientation*

Walls *Anisotropic Rigidity*

OpenAlea *Tissue Structure*

Initial structure



$$\mathbf{H}^{-1} : S_e = E_e$$

Mechanical equilibrium

computed through an implicit integration scheme

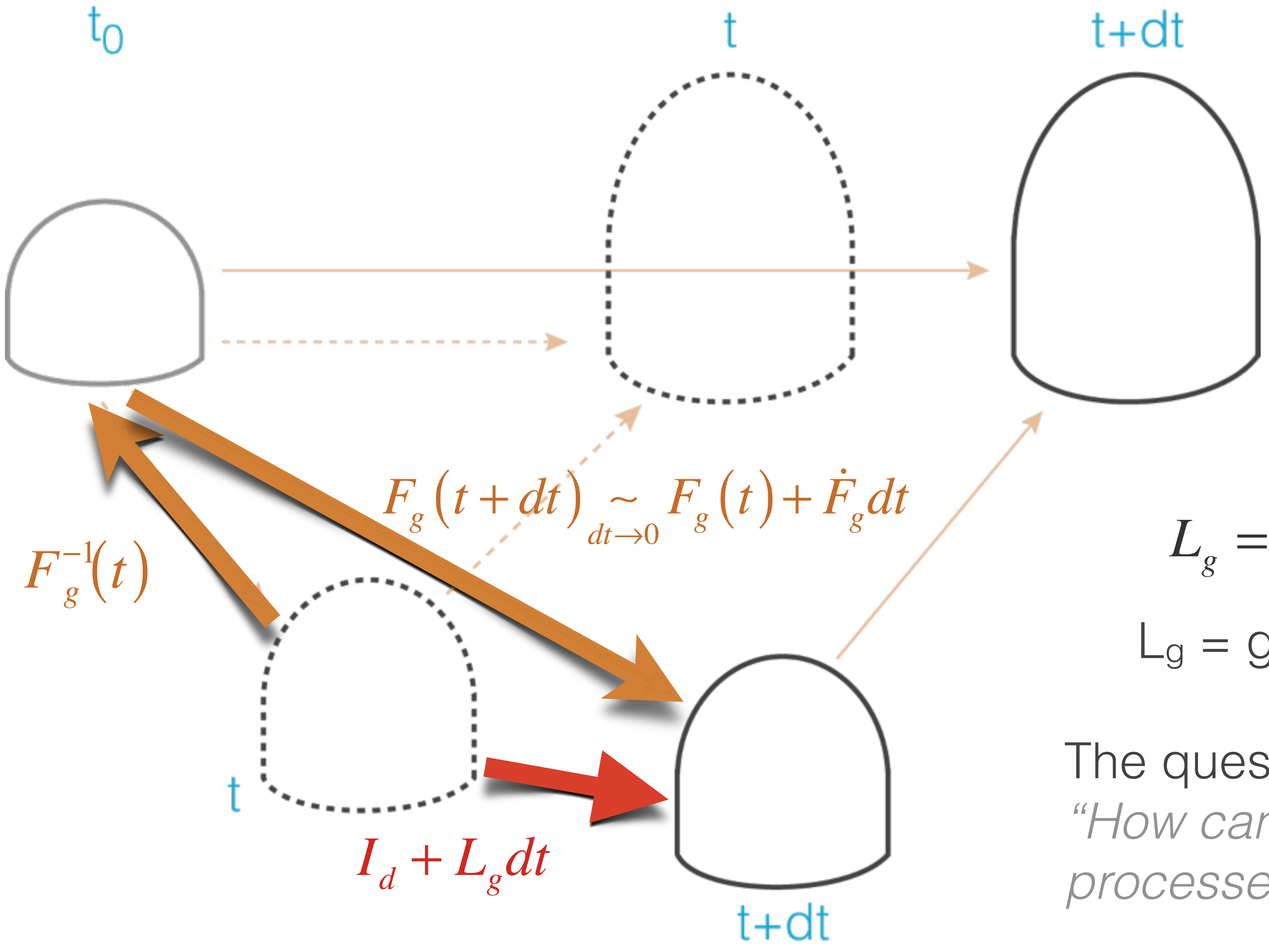
Output: E_e

Sofa *Mechanical Scene Graph*

Growth implementation

Growth implementation

Geometrical representation of growth



$$L_g = \dot{F}_g \cdot F_g^{-1} \quad \left(= \nabla_{X_g}\left(\vec{V}_g\right)\right) \qquad \left(\text{with} \quad \dot{F}_g = \frac{dF_g}{dt}\right)$$

L_g = growth velocity gradient

The question:

"How can we relate L_g to the actual biological processes ?"

Growth implementation

Phenomenological clues

$$L_g = ?\ldots$$

From experiments we know that:

1. *Growth is **fueled by** turgor **pressure***
2. *Turgor pressure has to reach a minimal **threshold** in order to induce growth*
3. *Growth is usually **perpendicular to CMFs'** main orientation.*

CMFs main direction = direction of highest rigidity

*3.bis Growth is usually **collinear to** elastic strain (**E_e**)*

$$L_g = \begin{cases} \gamma\left(E_e - E_{th}\right) & E_e \geq E_{th} \\ 0 & E_e \leq E_{th} \end{cases}$$

Growth implementation

Phenomenological growth law, toward a "Lockhart-like" equation

$$L_g = \gamma \left(E_e - E_{th} \right)$$

Extensibility

Threshold (*in strain*)

Linear Elasticity + Mechanical Equilibrium:

$$E_e = \mathbf{C} : S_e \left(P \right)$$

With the compliance: $\mathbf{C} = \mathbf{H}^{-1}$

$$L_g = \Gamma : \left(S_e \left(P \right) - S_{th} \right)$$

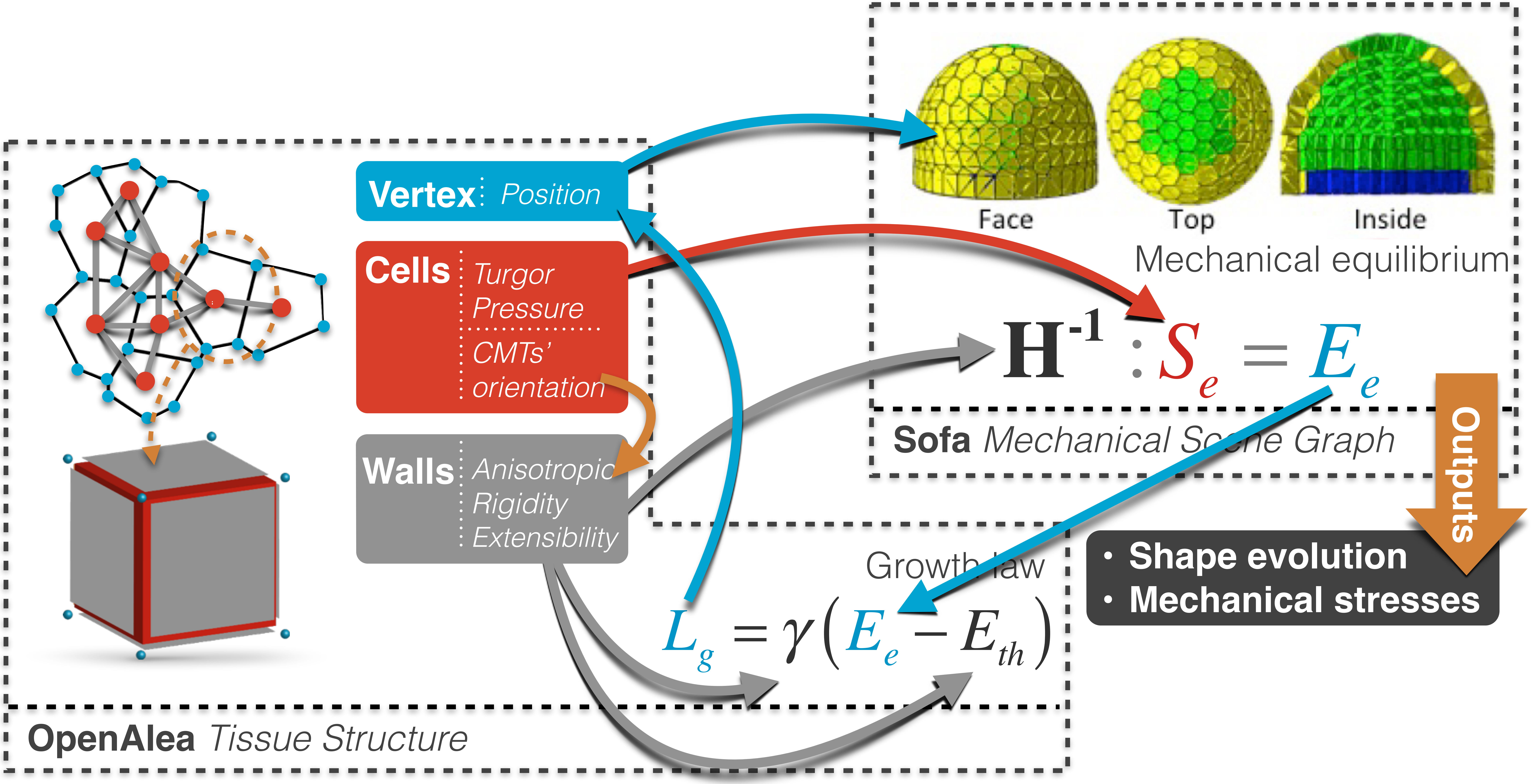
With: $\begin{cases} \Gamma = \gamma \mathbf{C} \\ S_{th} = \mathbf{H} : E_{th} \end{cases}$

Tensorial extensibility

Threshold (*in stress*)

Intermediate sum up (II)

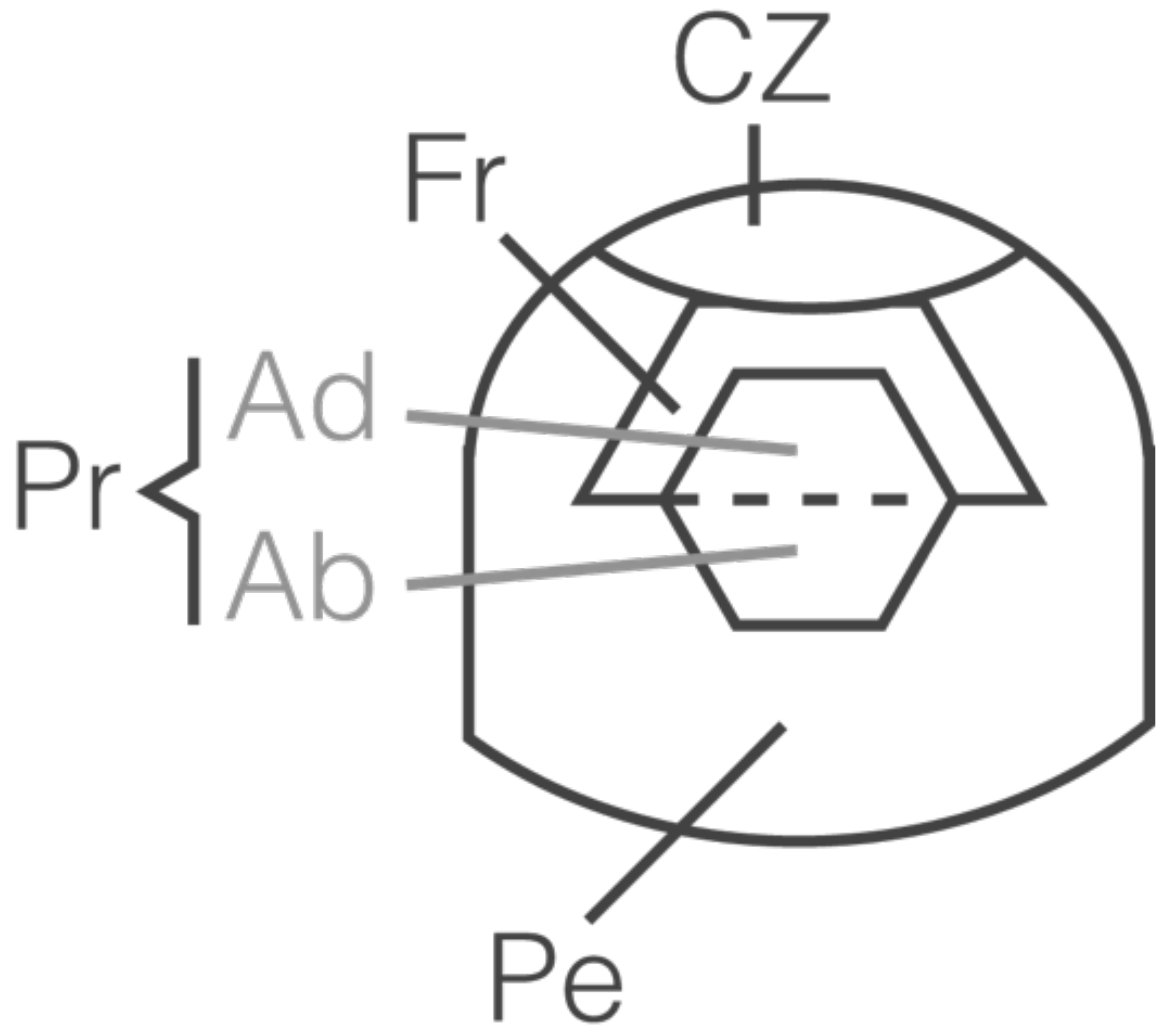
The feed-back loop is completed by the growth equation

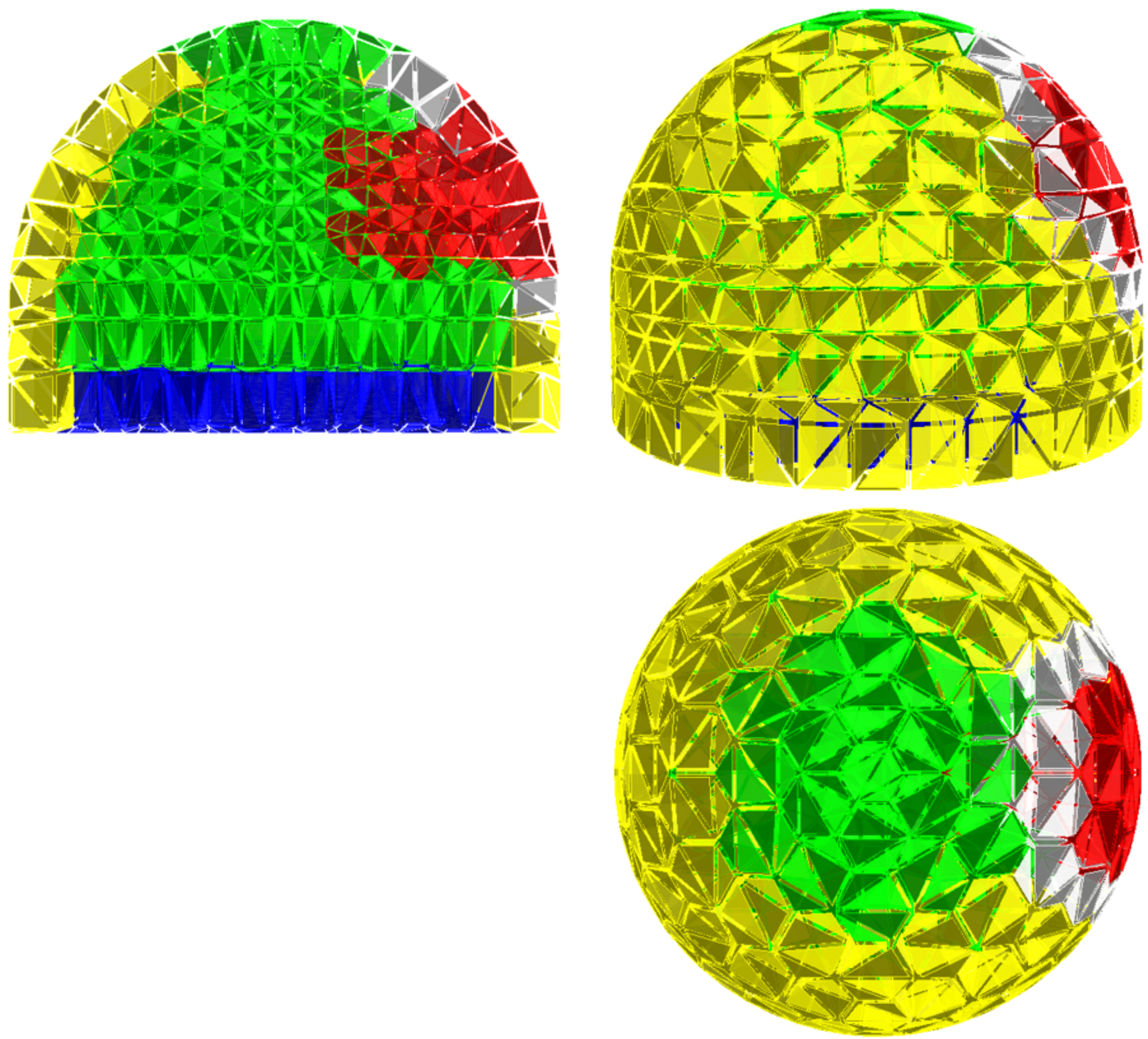


Some qualitative results

Purely digital structure

The i-meristem…



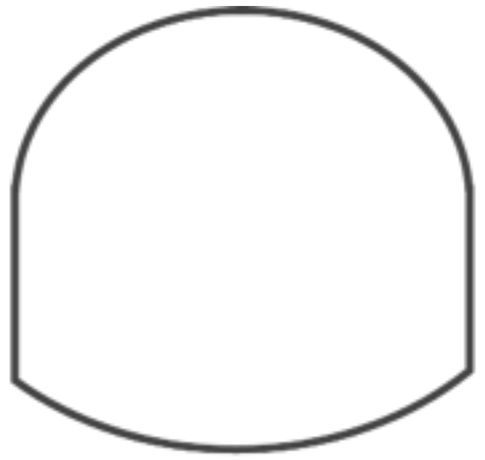


- CZ: Central Zone
- Fr: Frontier
- Pr: Primordium
  - Ad: Adaxial zone
  - Ab: Abaxial zone
- Pe: Periphery

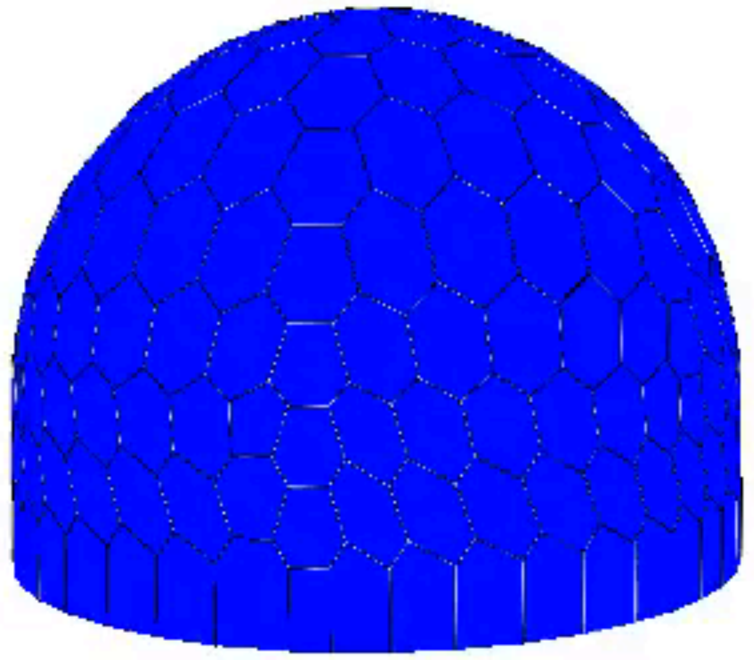
First simulations: How to get directional growth

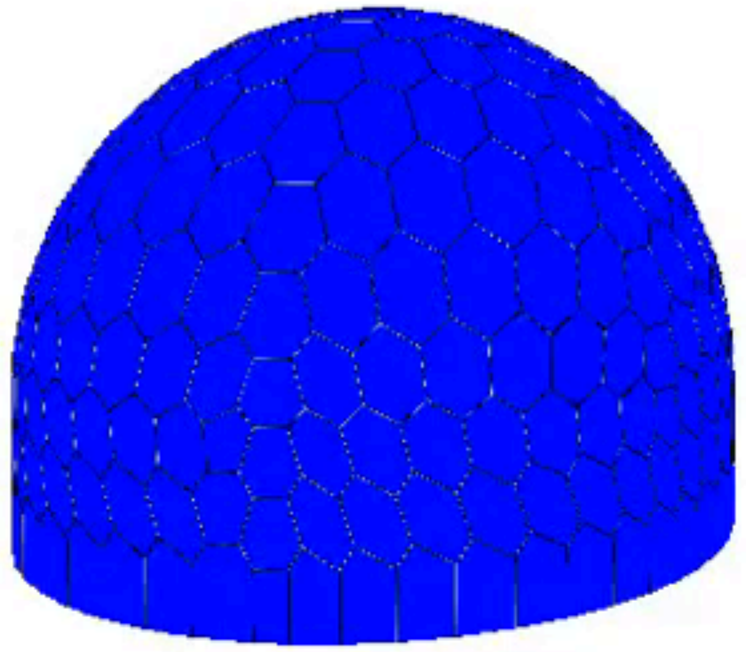
Anisotropic rigidity is required to sustain directional growth

2 zones:

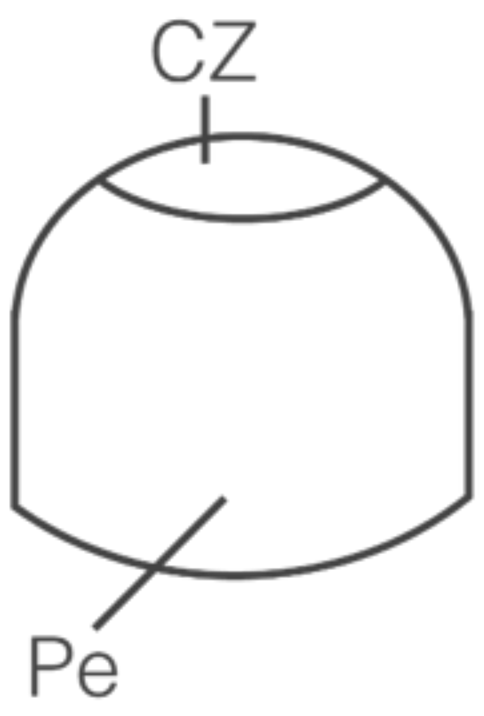


The whole structure: Homogenous & isotropic





Central Zone: isotropic rigidity



Periphery: anisotropic rigidity

Sassi et al, Current Biol. 2014

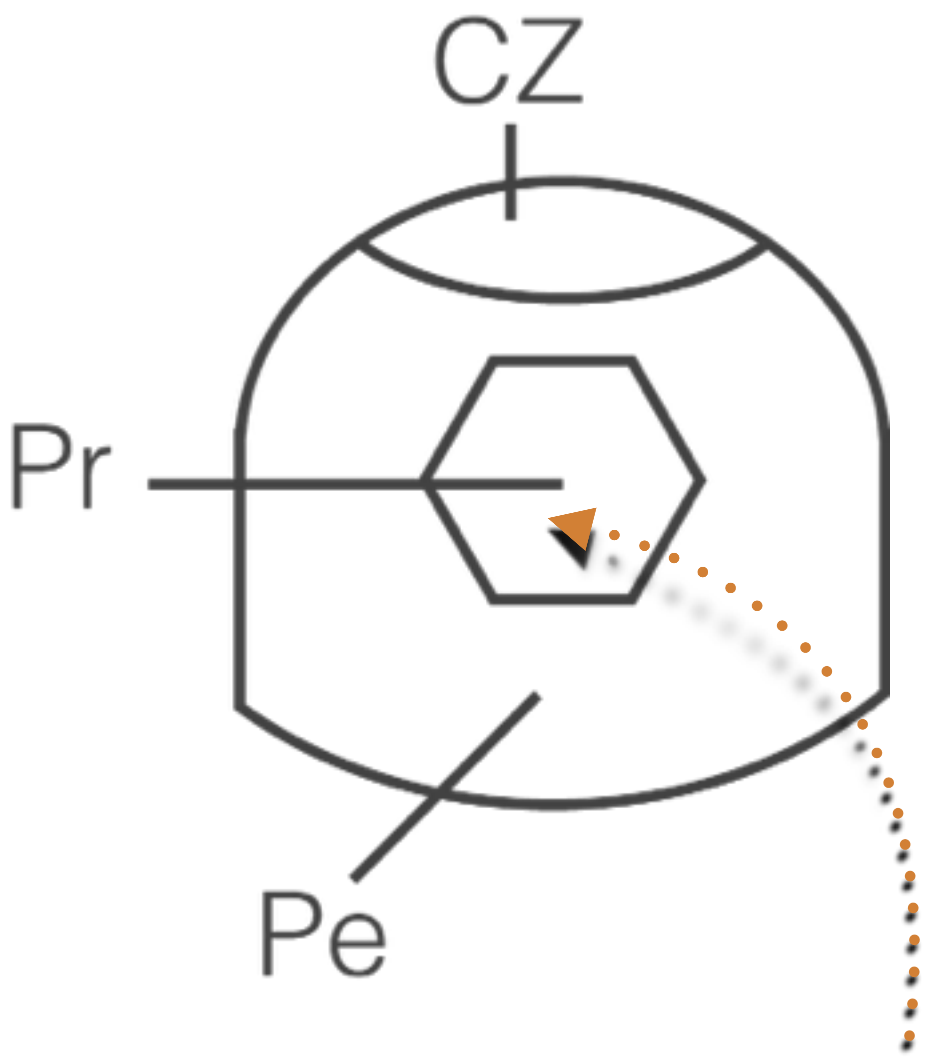
Boudon, Ali, Chopard et al., submitted

Mechanically isotropic structures evolves toward spheroid structures

In order to generate directional growth several strategies could be applied

Primordium initiation - I

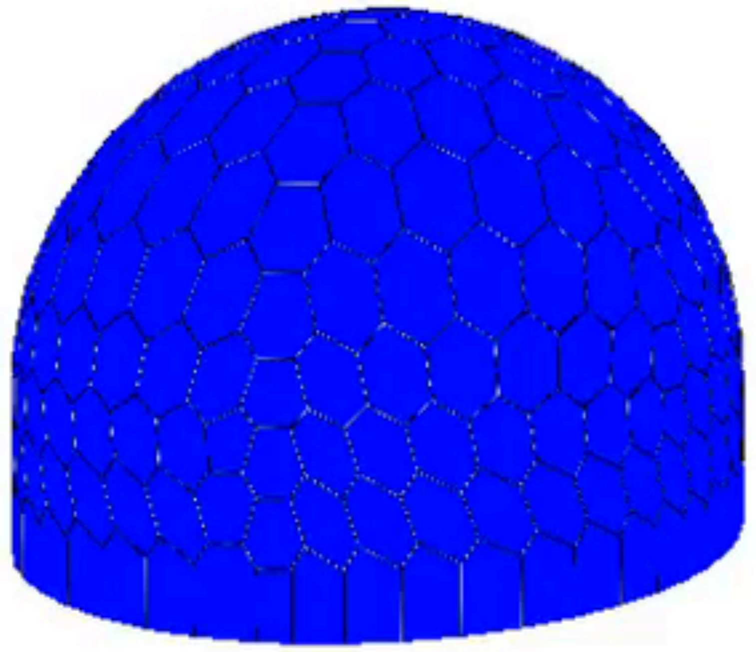
Softening of the initial seems to be the first step in the generation of a lateral organ



Softening the cell wall means: $\searrow \mathbf{H}$

$$L_g = \Gamma : \left(S_e(P) - S_{th} \right)$$

$$\Gamma = \gamma \mathbf{C} \nearrow \qquad S_{th} = \mathbf{H} : E_{th} \searrow$$



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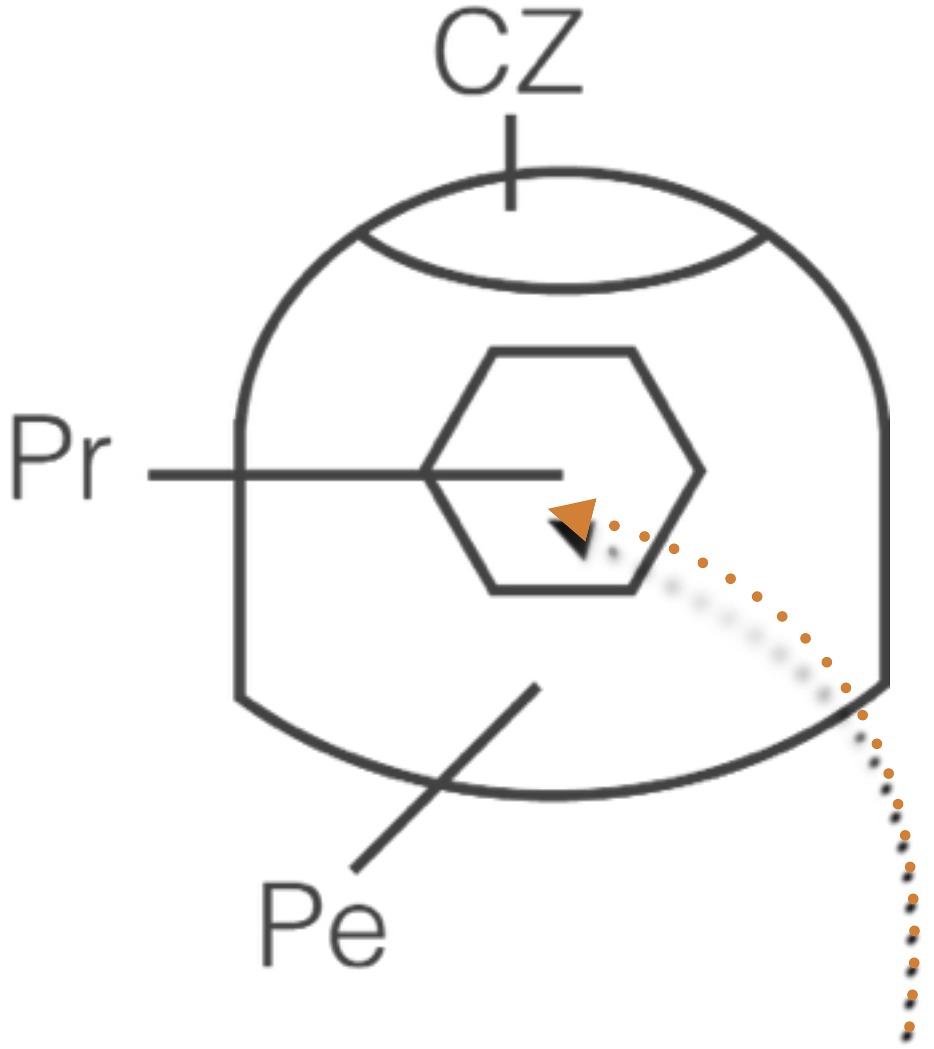

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Current Biology *21*, 1720–1726, October 25, 2011

Pectin-Induced Changes in Cell Wall Mechanics Underlie Organ Initiation in *Arabidopsis*

Alexis Peaucelle,[1,2,5,*] Siobhan A. Braybrook,[3,5] Laurent Le Guillou,[4] Emeric Bron,[2,4] Cris Kuhlemeier,[3] and Herman Höfte[1,*]

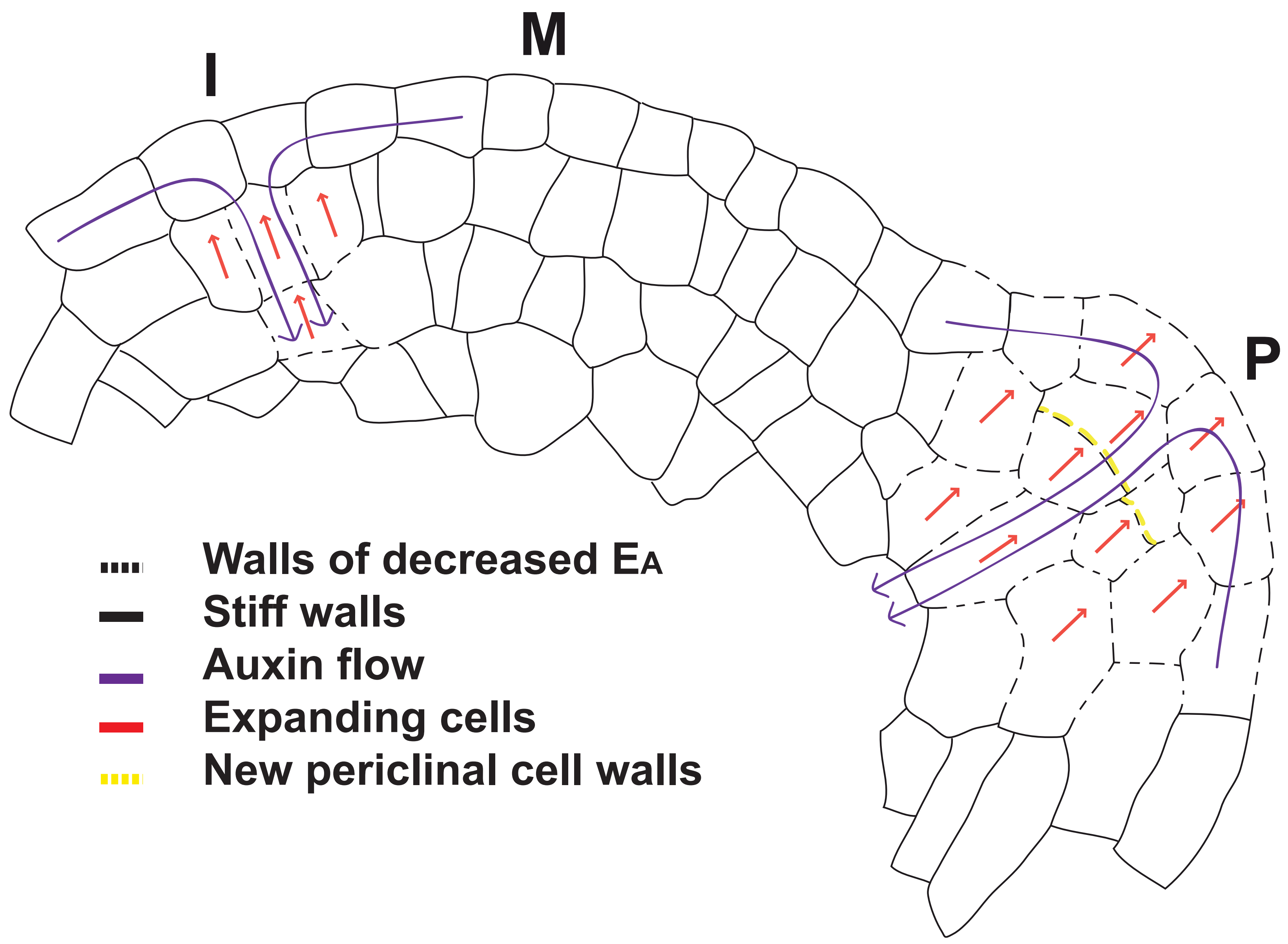

Figure 4. Organogenesis in *Arabidopsis* from a Mechanochemical Perspective

Representation of an *Arabidopsis* inflorescence meristem. Auxin flow creates maxima at incipient organ sites; purple arrows show the direction of auxin flow in the outer epidermal layer (L1) as well as the channeling down of auxin at the convergence point. At these positions, localized de-methylesterification of pectins in the cell walls occurs, which contributes to increases in wall elasticity (a decrease in the apparent Young's modulus, E_A). The decrease in E_A is the first observable mechanical event associated with cell expansion driving organ emergence (red arrows). At the site of an incipient organ (I), the decrease in E_A is detected only in the underlying tissues, not in the epidermal layer, illustrating that the increase in wall elasticity occurs first in subepidermal layers. Within an emerged organ (P), the walls of all cells have a lower E_A, which is correlated with the increased growth rate of these cells. The decrease in E_A in underlying tissues first could lead to periclinal divisions in the L2 (yellow lines) as it diverges from a distinct monolayer of cells into a more complex tissue. Within the meristem dome (M), all cells appear to have relatively stiffer walls.

Primordium initiation - I

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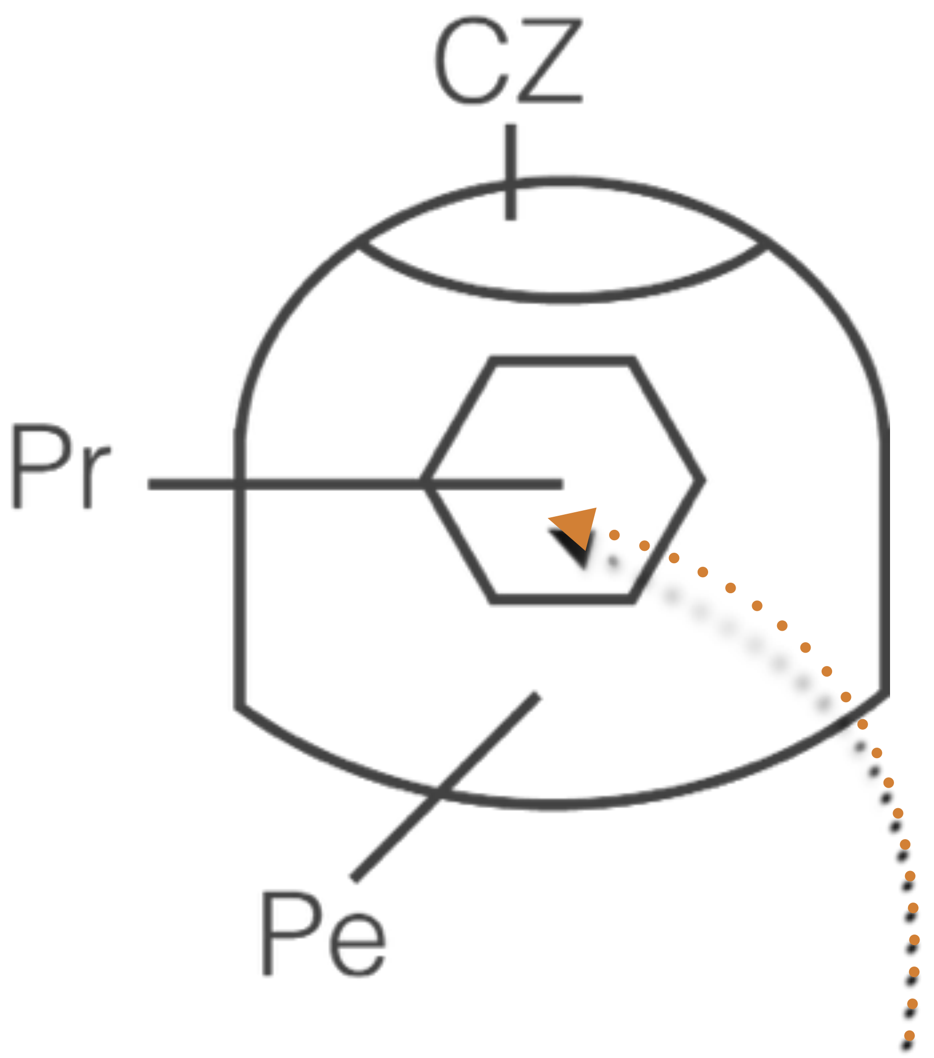

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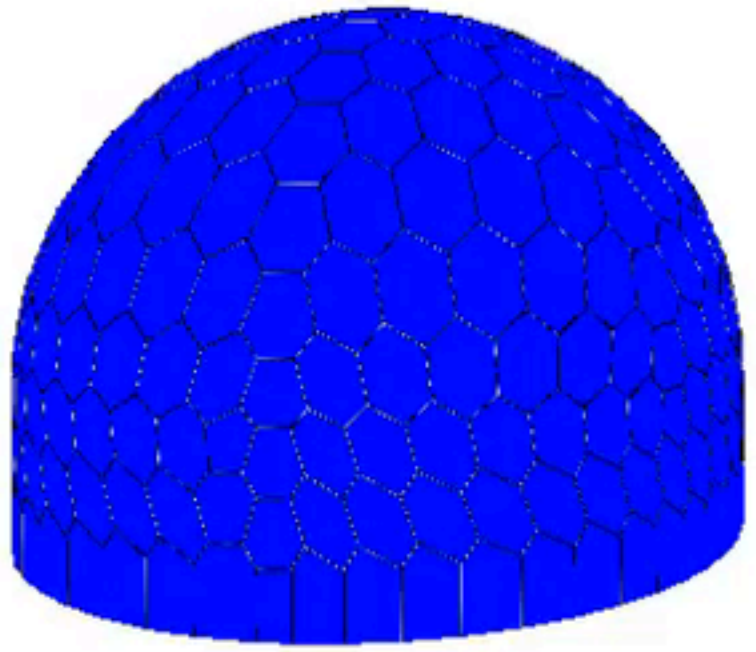
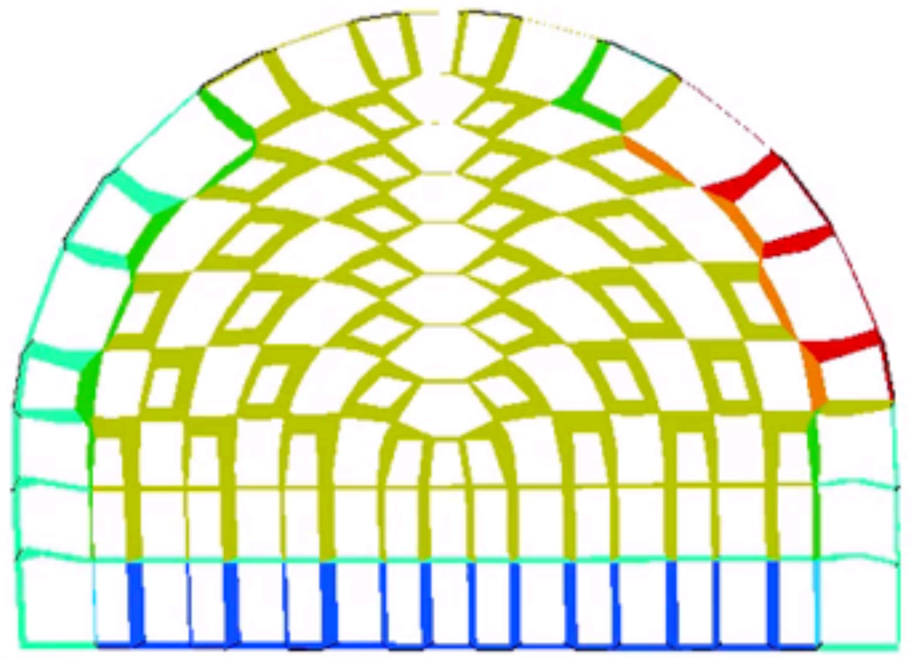
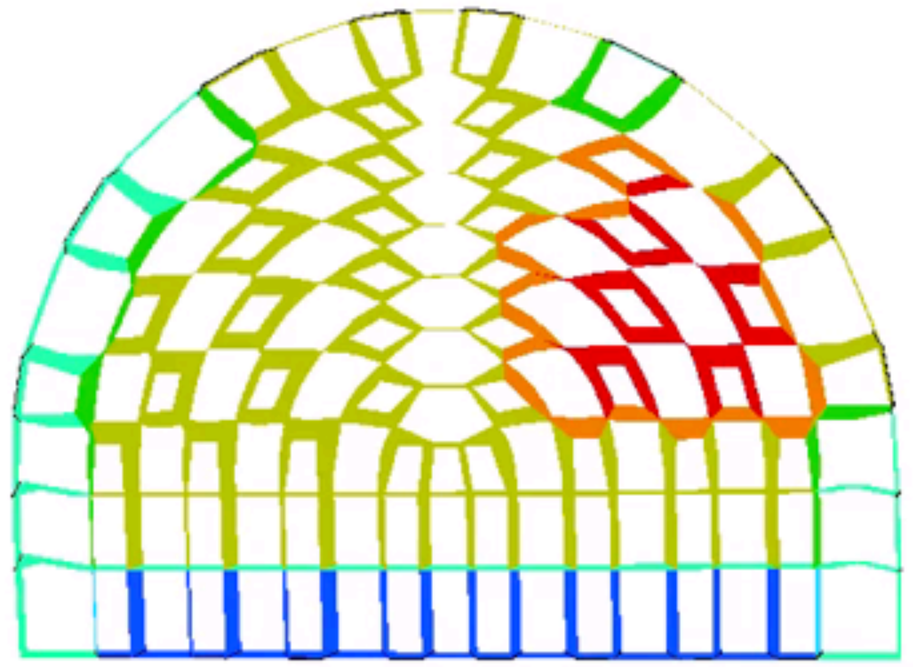


Boudon, Ali, Chopard et al., submitted

Inner view:



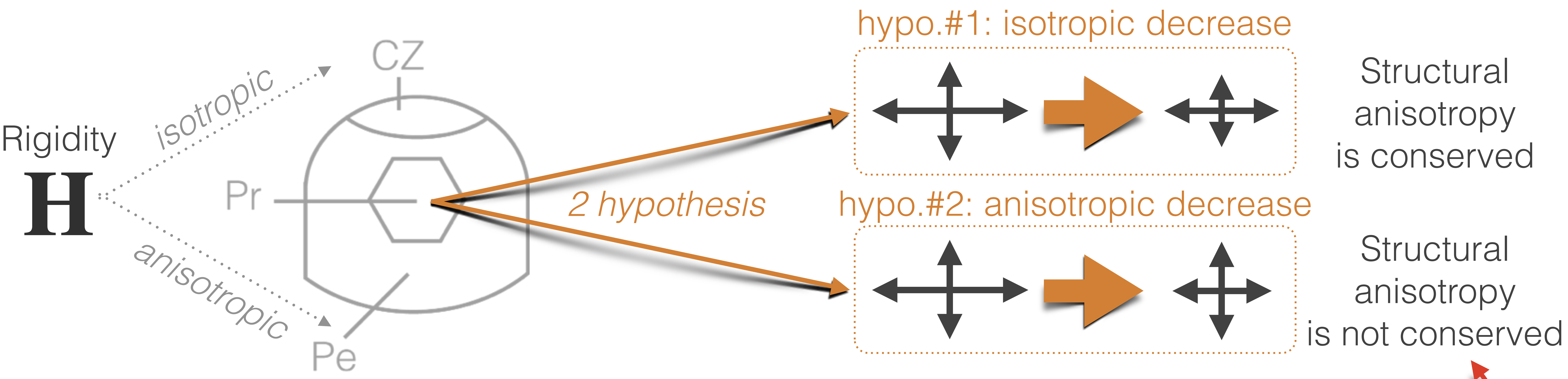
Softening of the L_1 layer

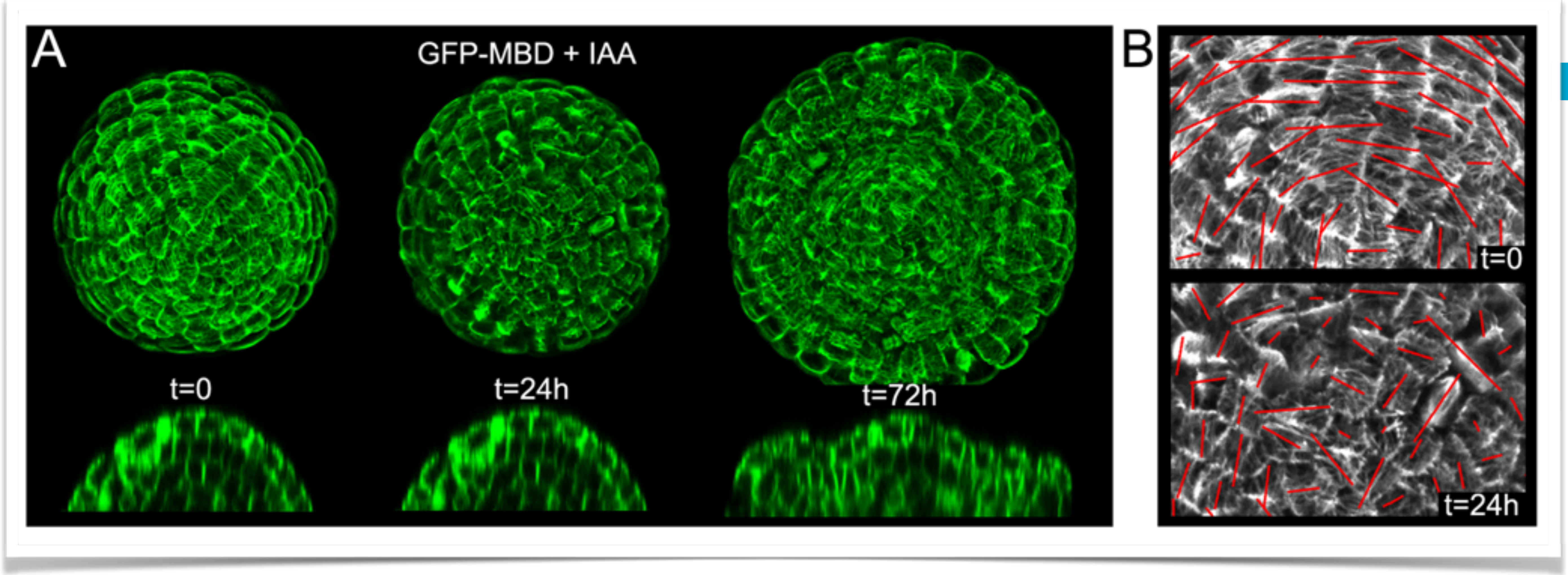


Softening of the inner tissue

Primordium initiation - II

How does "softening of the cell wall" happen ?





Sassi et al, Current Biol. 2014

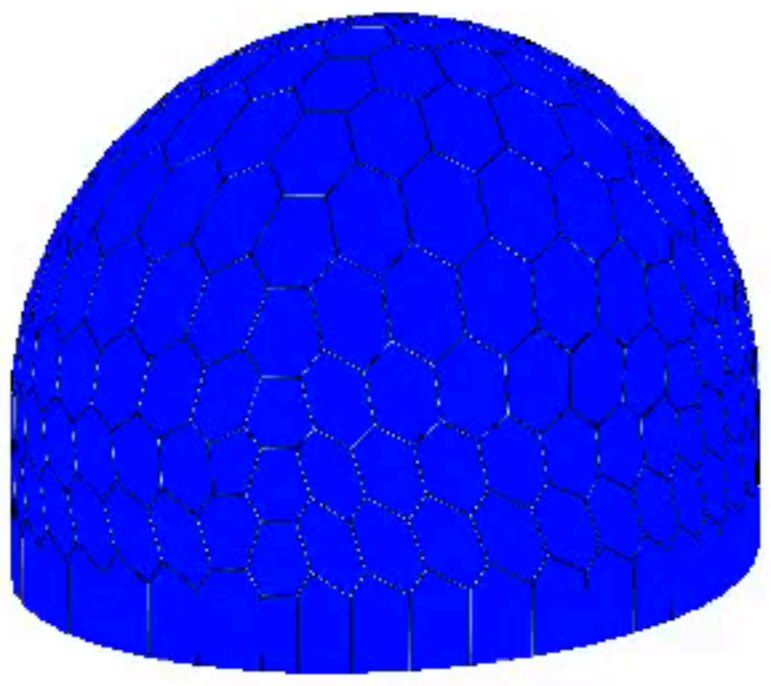
First event:
MicroTubules disorganization

Csquence:
Partial Loss of structural anisotropy

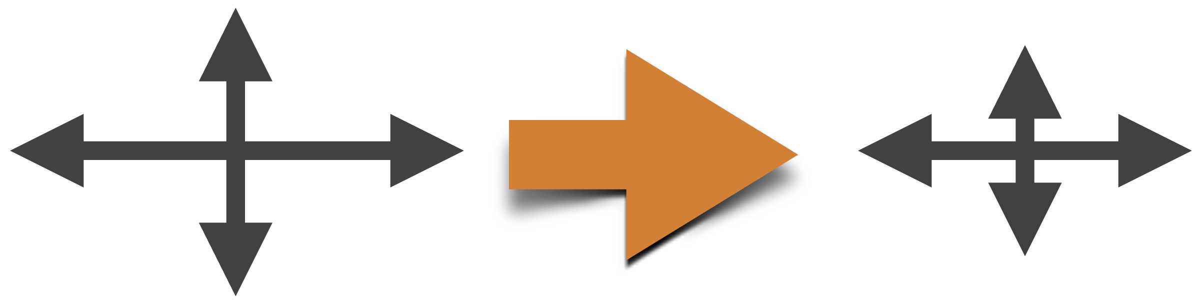
Primordium initiation - II

Does a loss of structural anisotropy is required to initiate lateral organs ?

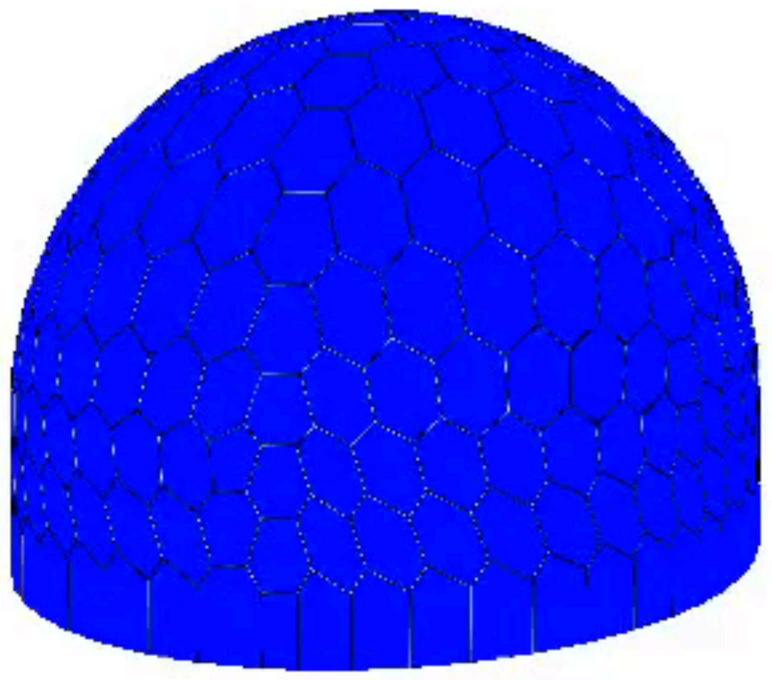
Sassi et al, Current Biol. 2014



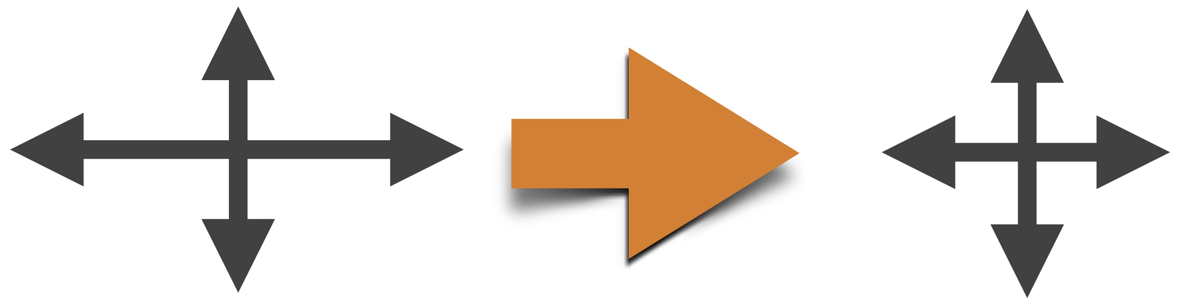
hypo.#1: isotropic decrease



Structural anisotropy is conserved



hypo.#2: anisotropic decrease

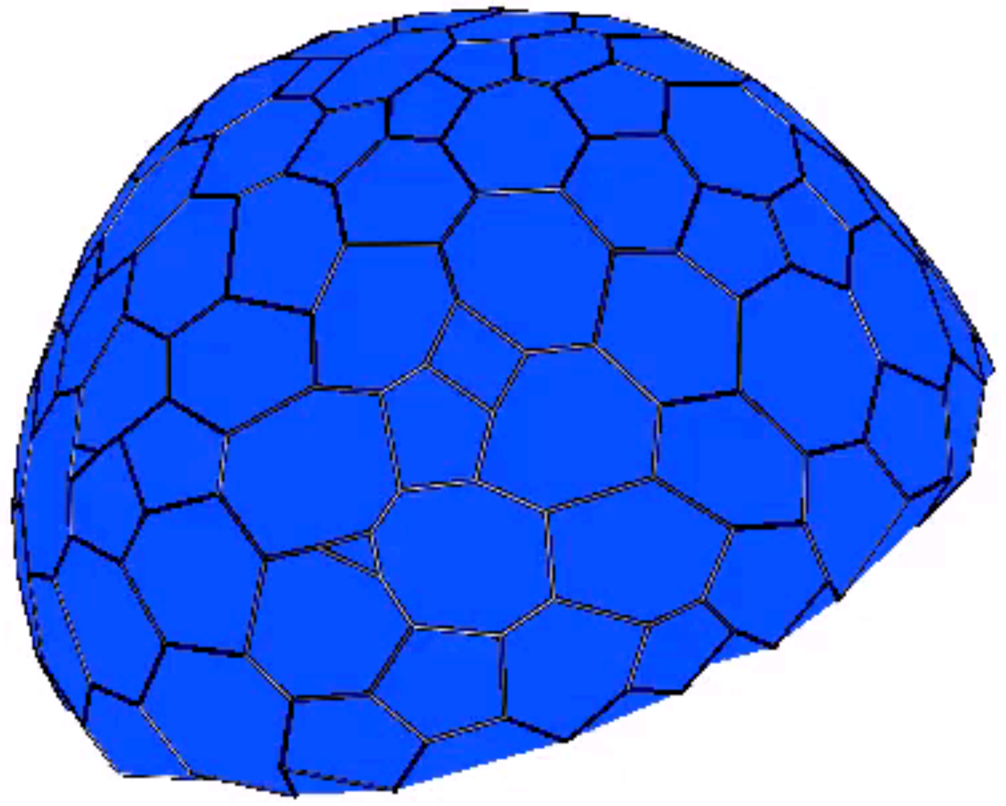


Structural anisotropy is not conserved

Loss of structural anisotropy is required but not sufficient - ↘ *of the overall rigidity is also needed*

Flower bud formation

Toward more complex shapes generation



Initial conditions:

- Digitalized structure
- Differentiation pattern

Translated into mechanical characteristics

+

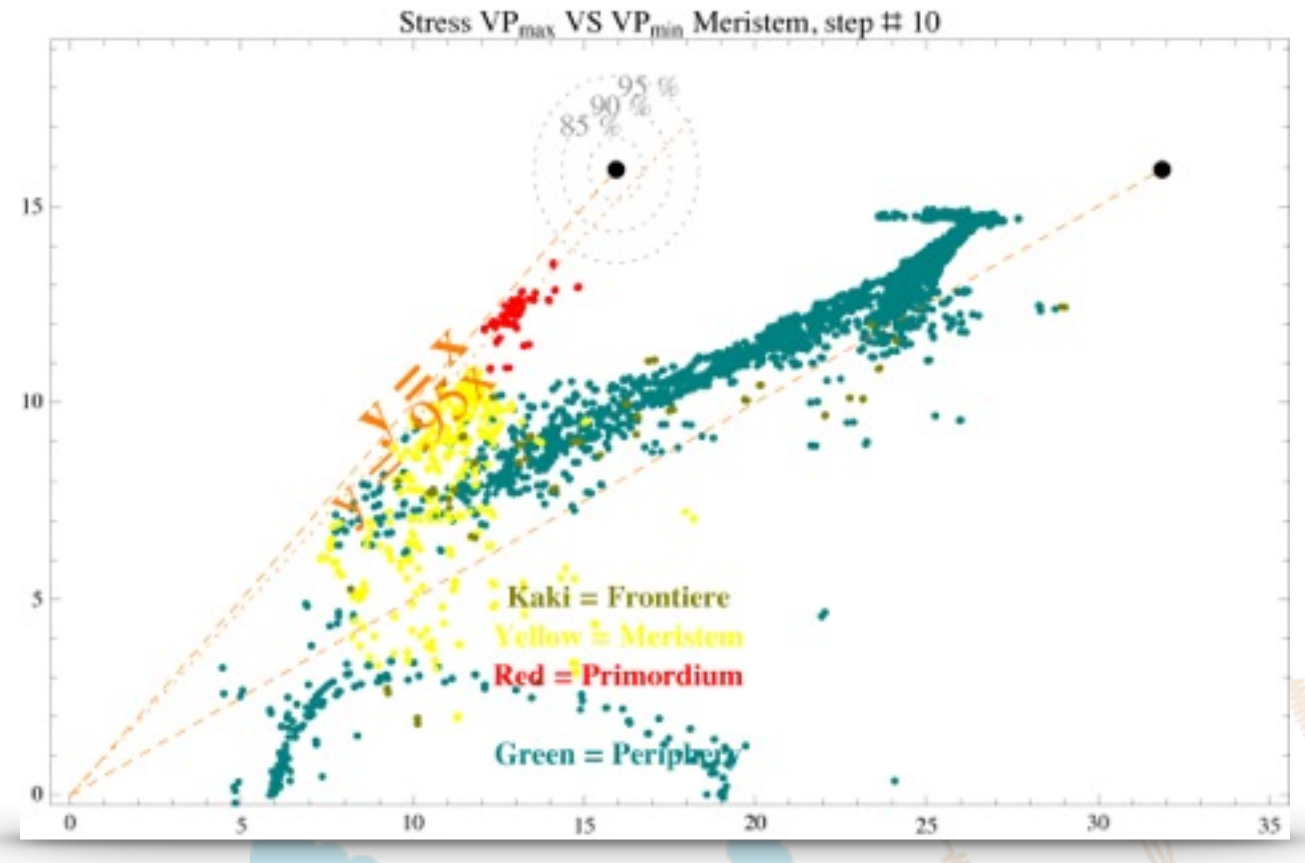
Cell division !
(work in progress)

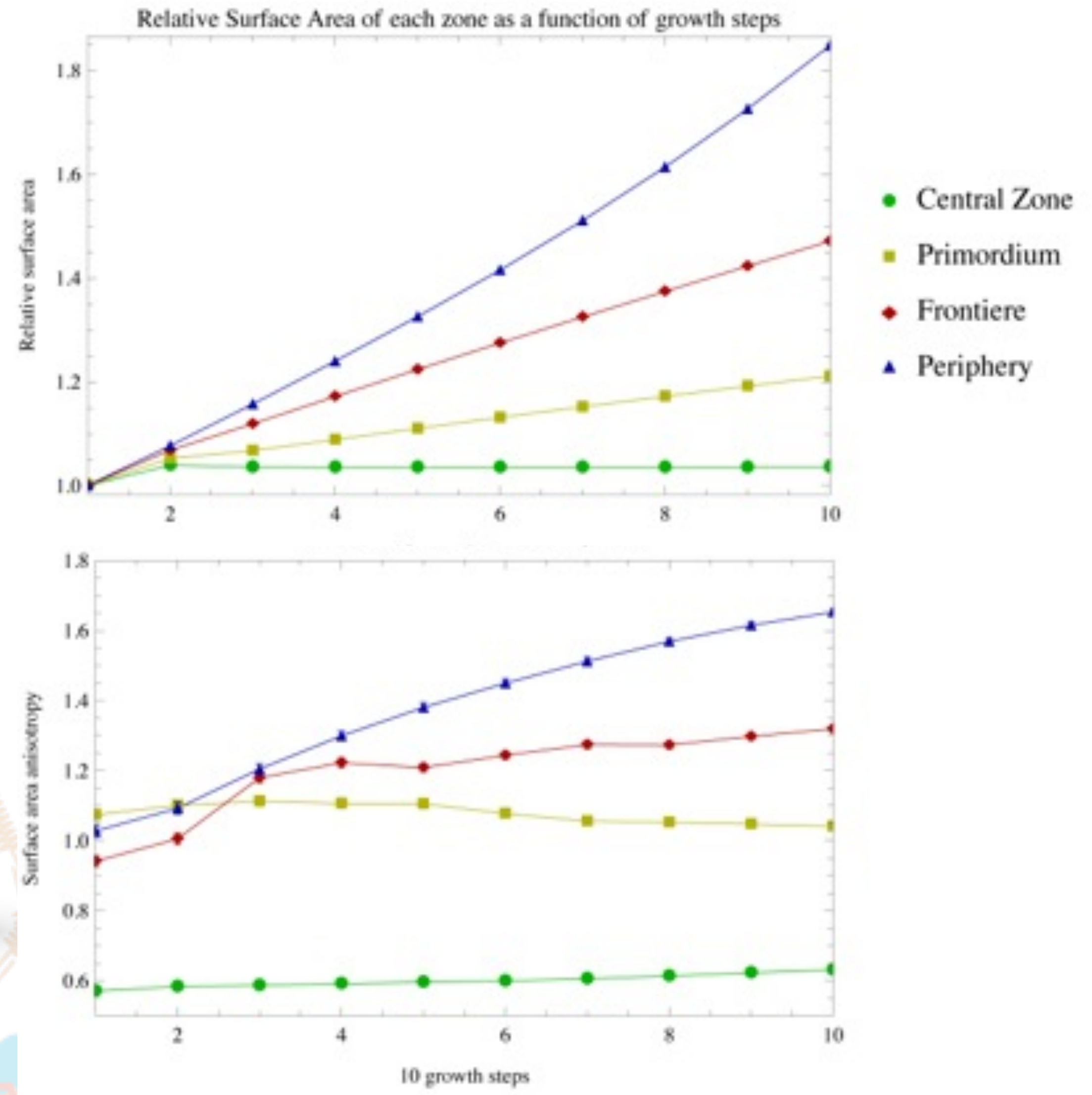
What's next ?

. . .

In terms of numerical framework development:

- Cell division implementation
- Quantitative outputs





In terms of physics & modelling:

- Molecular grounds for the phenomenological growth equation
- Riemannian geometry formalism… (?)

$$r_g = \gamma \cdot \left(f_{gel} - f_{th} \right)$$

In terms of biology:

- Cell wall structural properties visualization
- Interactome of cell wall modifying enzymes

Thanks !

Collaborators on the project

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