Genomics, Epigenetics & Synthetic Biology

Lecture 4: Self-organisation and reprogramming of multicellular systems

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www.haseloff-lab.org

Application of Synthetic Biology

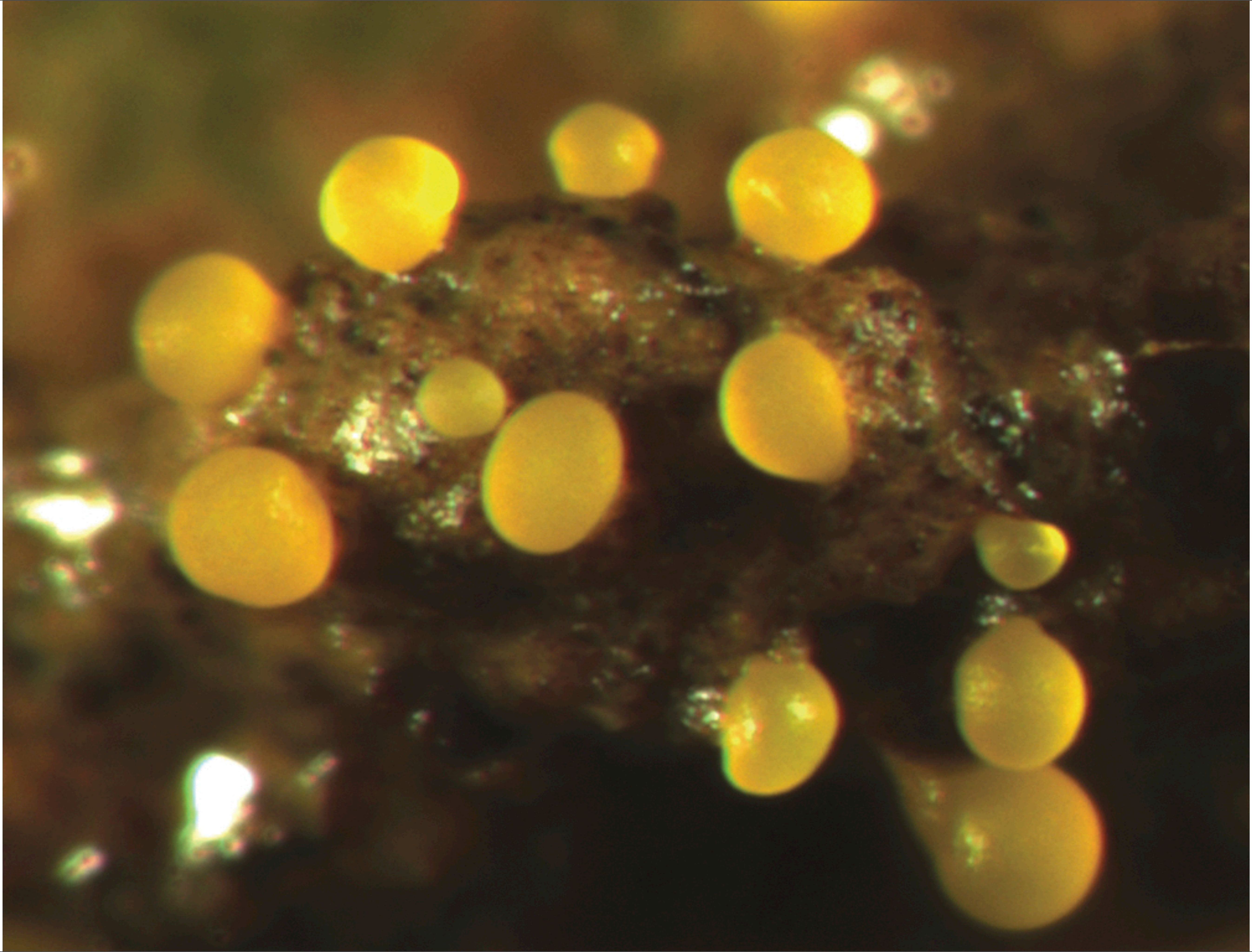
1. Cell autonomous genetic circuits with self-regulating properties

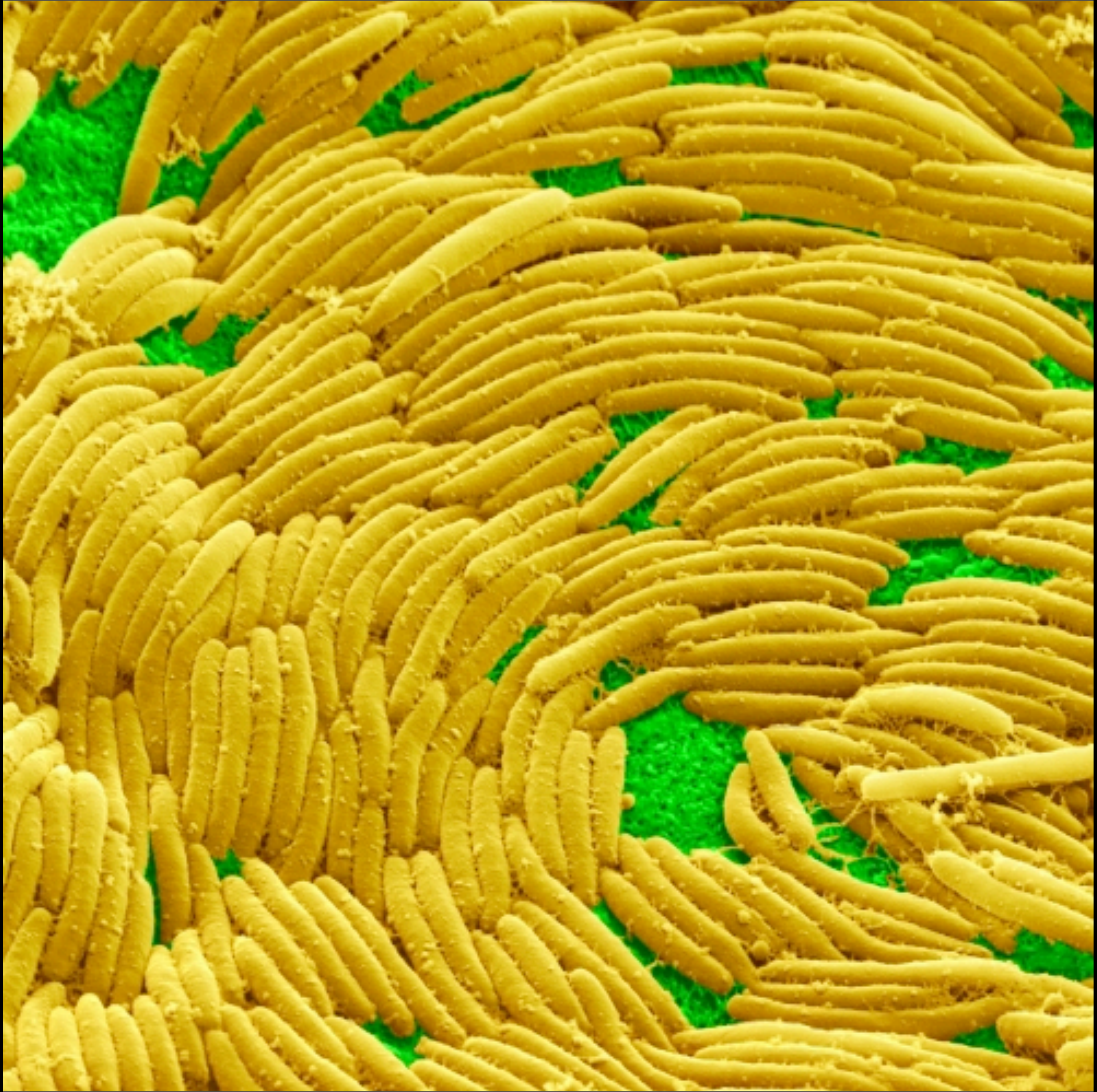
e.g. microbial engineering,
enviromental and biomedical sensors
engineering novel metabolic pathways

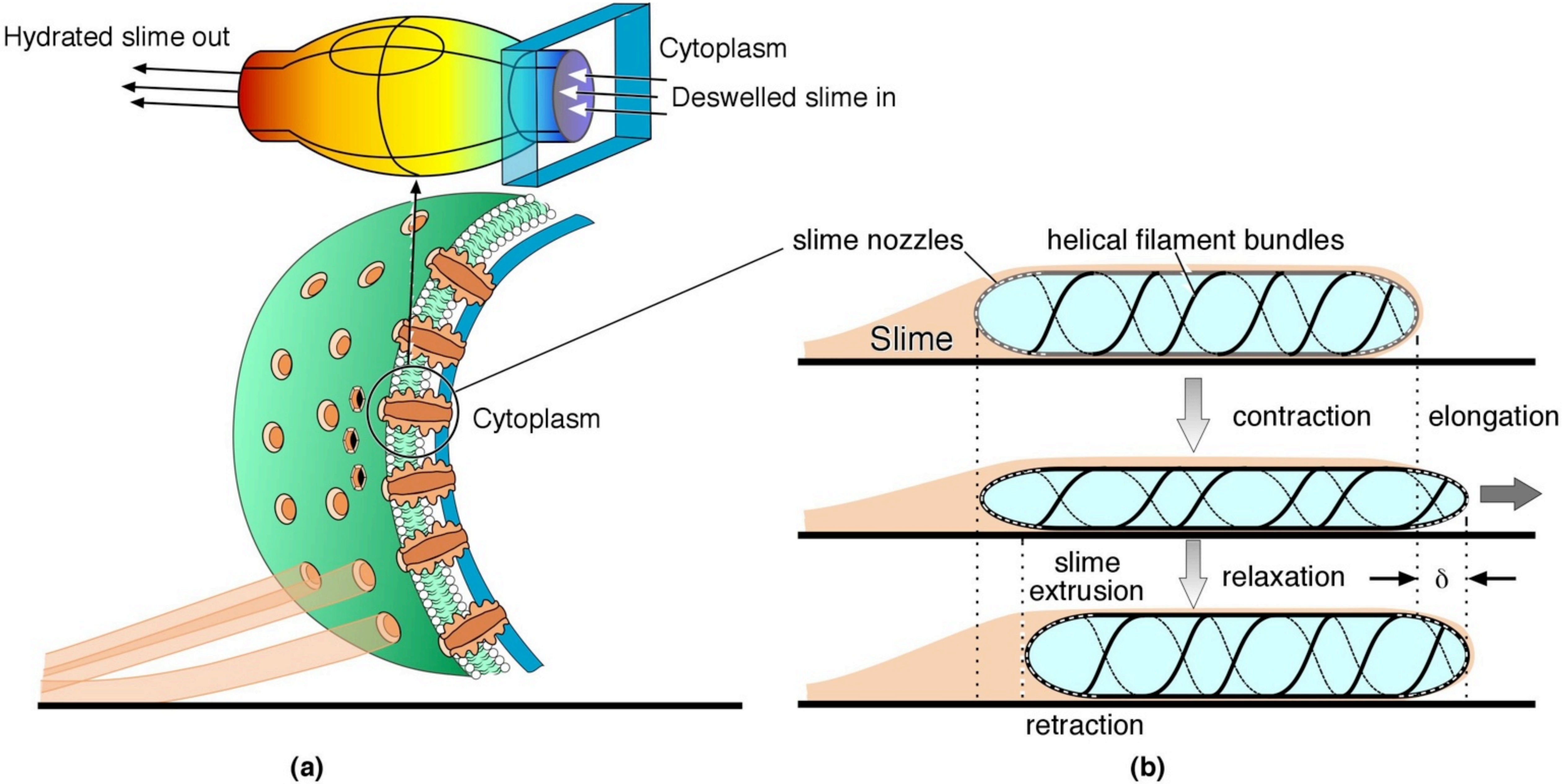
2. Morphogenetic circuits with self organising properties

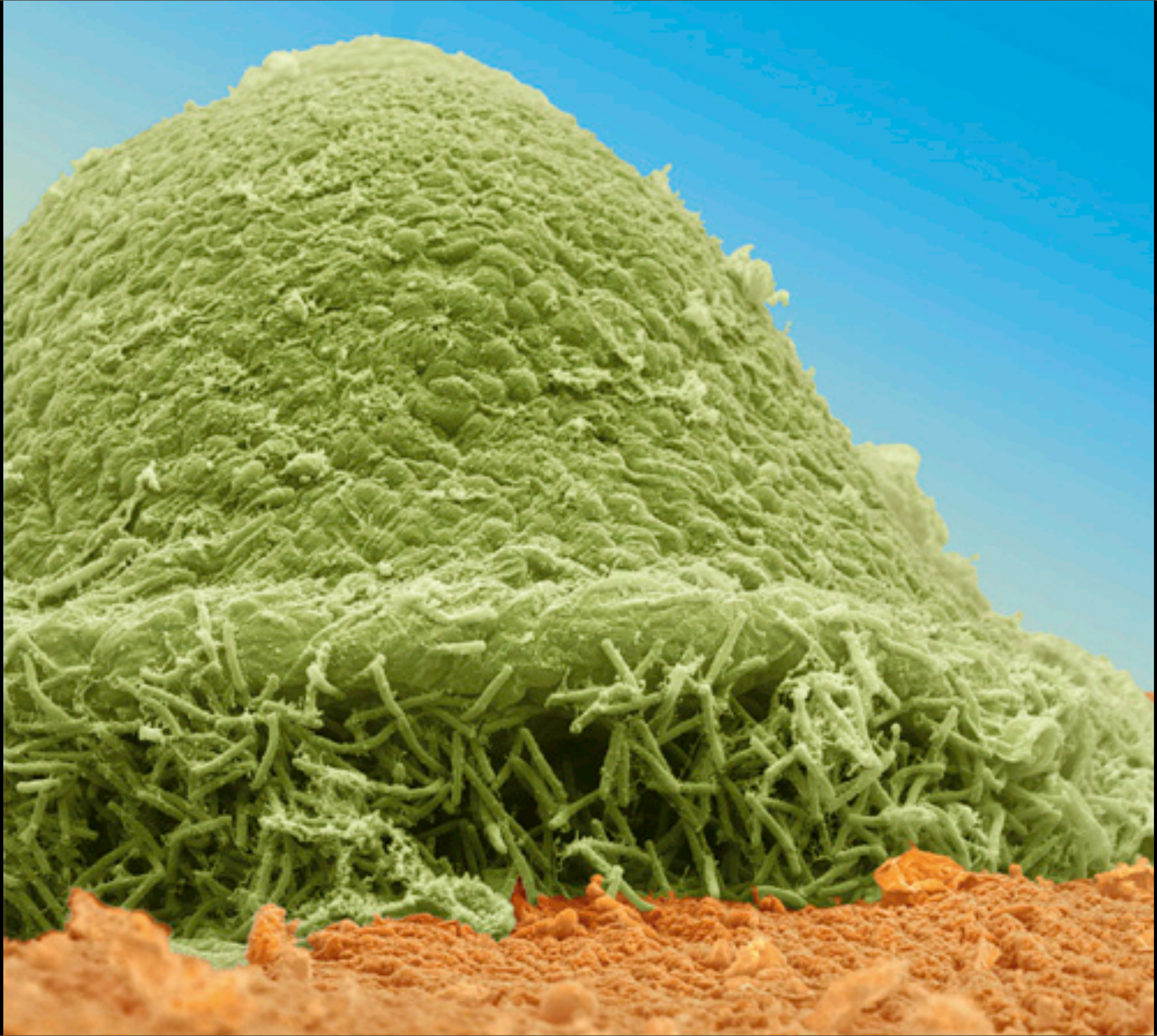
e.g. microbial biofilms or self-organising communities for
bioremediation and bio catalysis
novel plant and algal feedstocks for bioproduction and bioenergy
tissue engineering

Self-organisation at the cellular scale

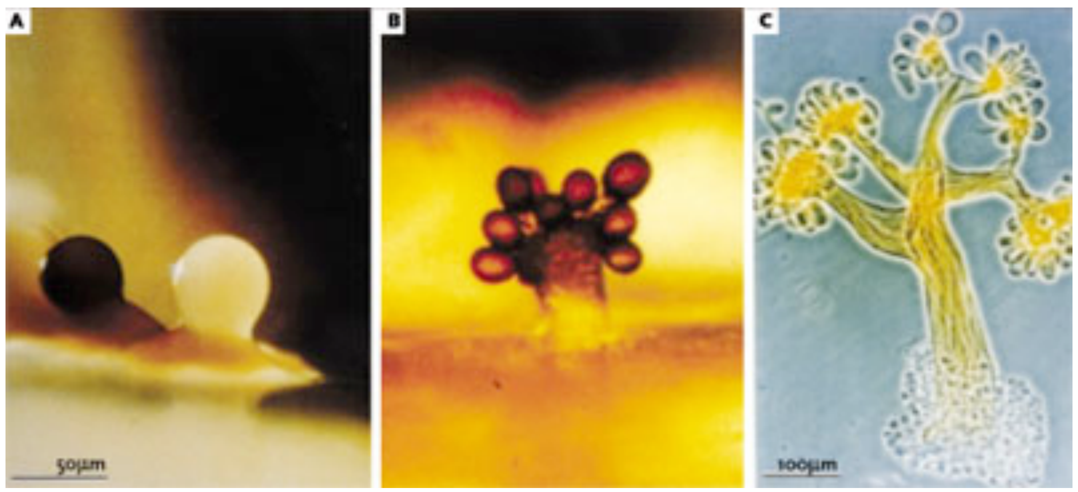


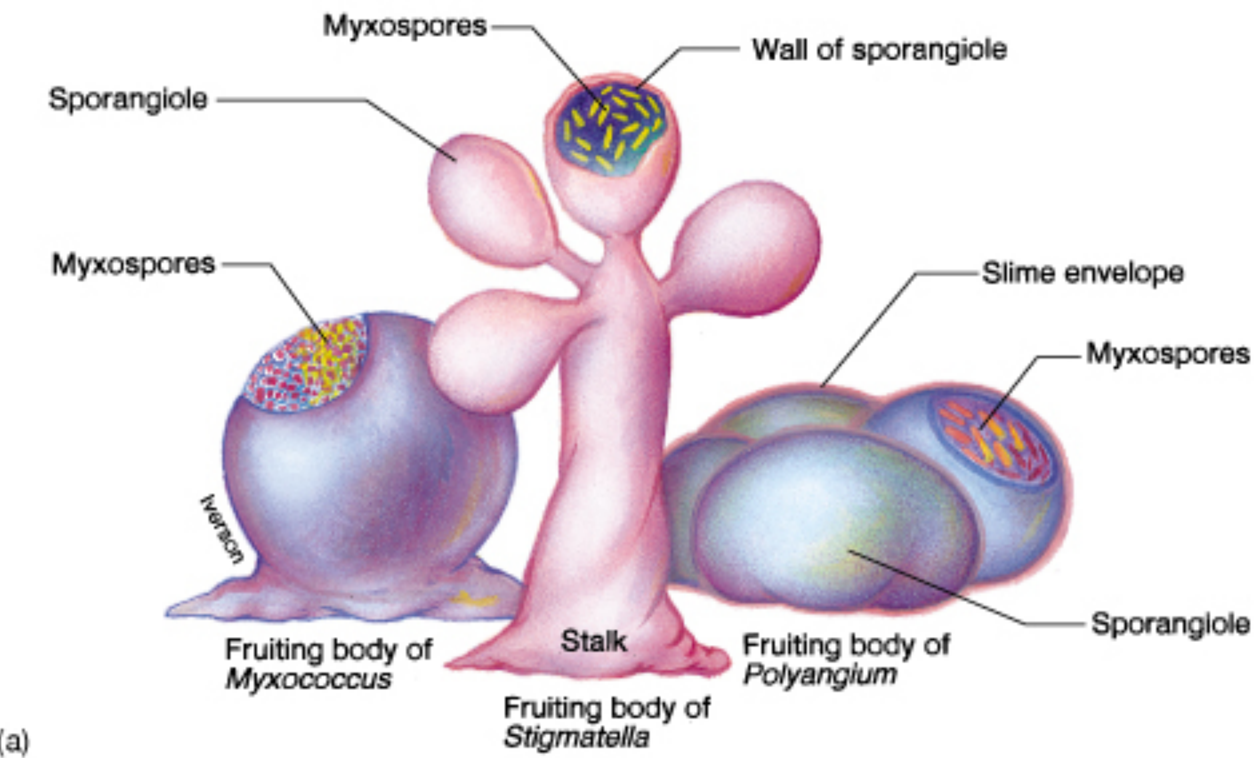



Hydrated slime out
Cytoplasm
Deswelled slime in
Cytoplasm
slime nozzles
helical filament bundles
Slime
contraction
elongation
slime
extrusion
relaxation
δ
retraction
(a)
(b)

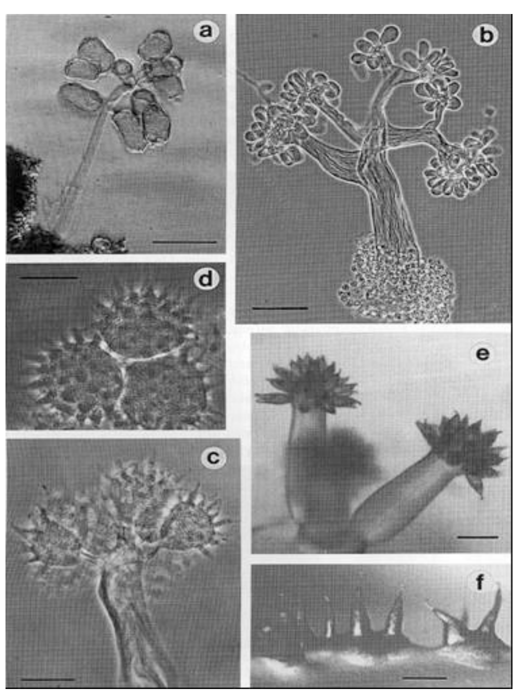


Morphogenetic bacteria





(a)



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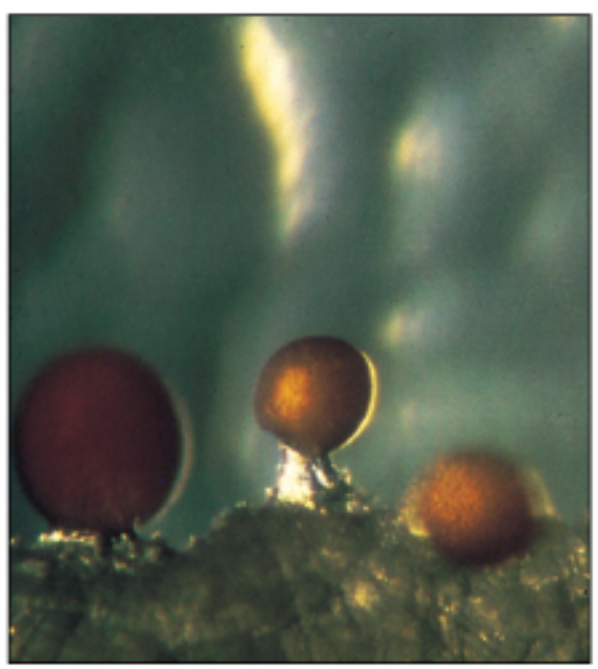
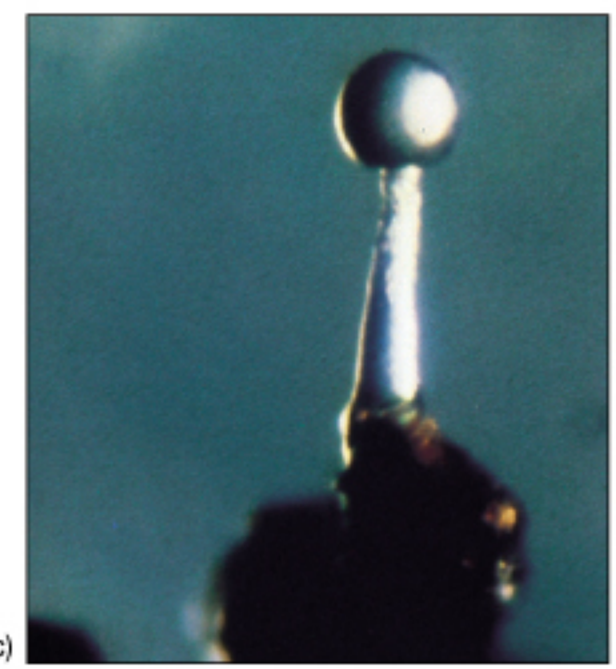
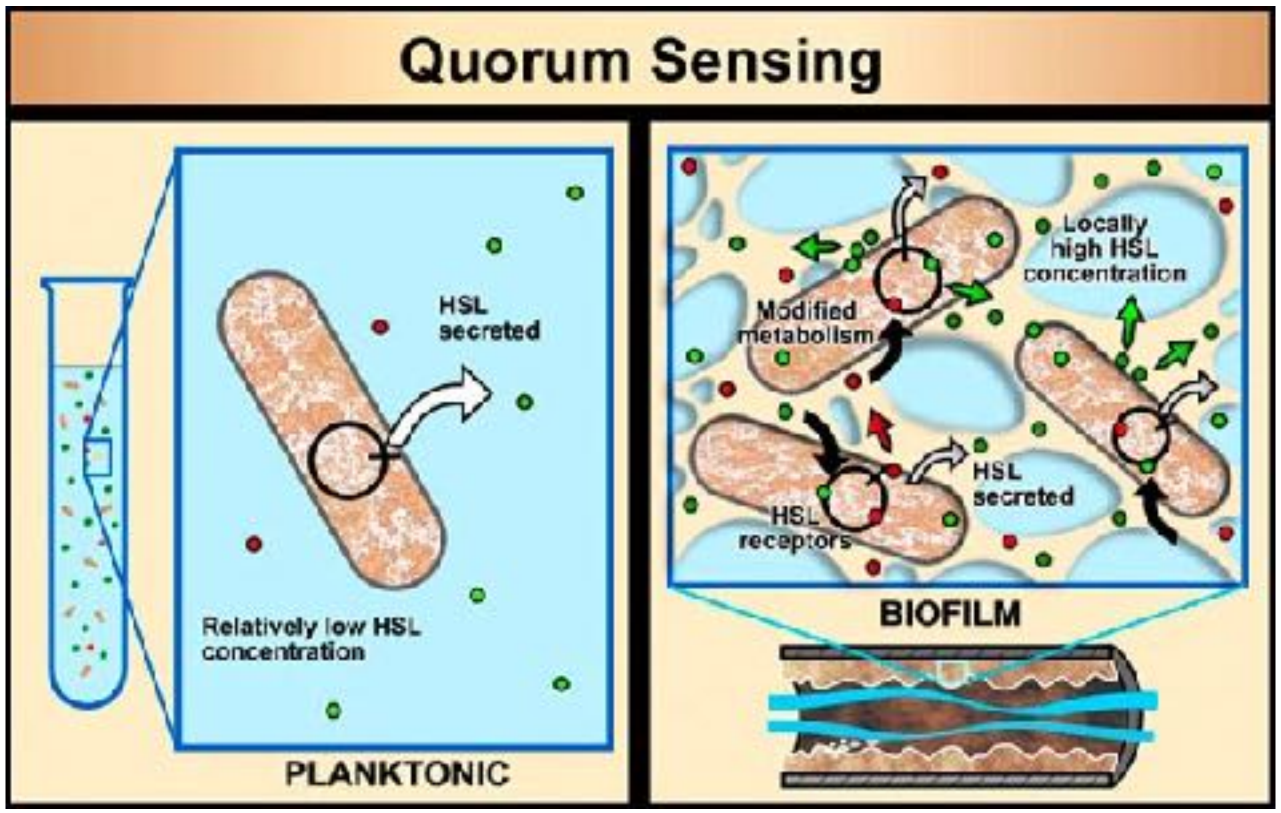


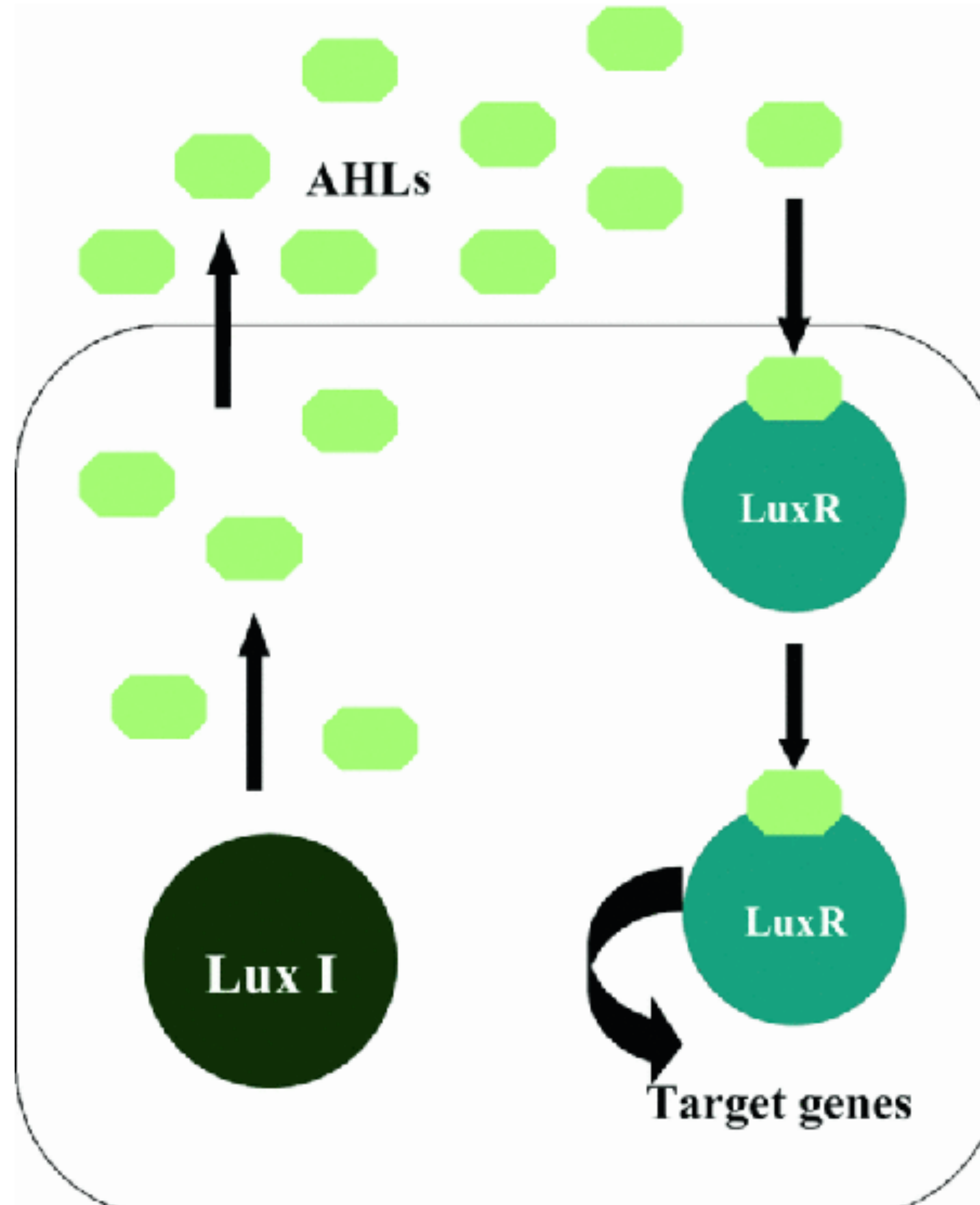
(c)



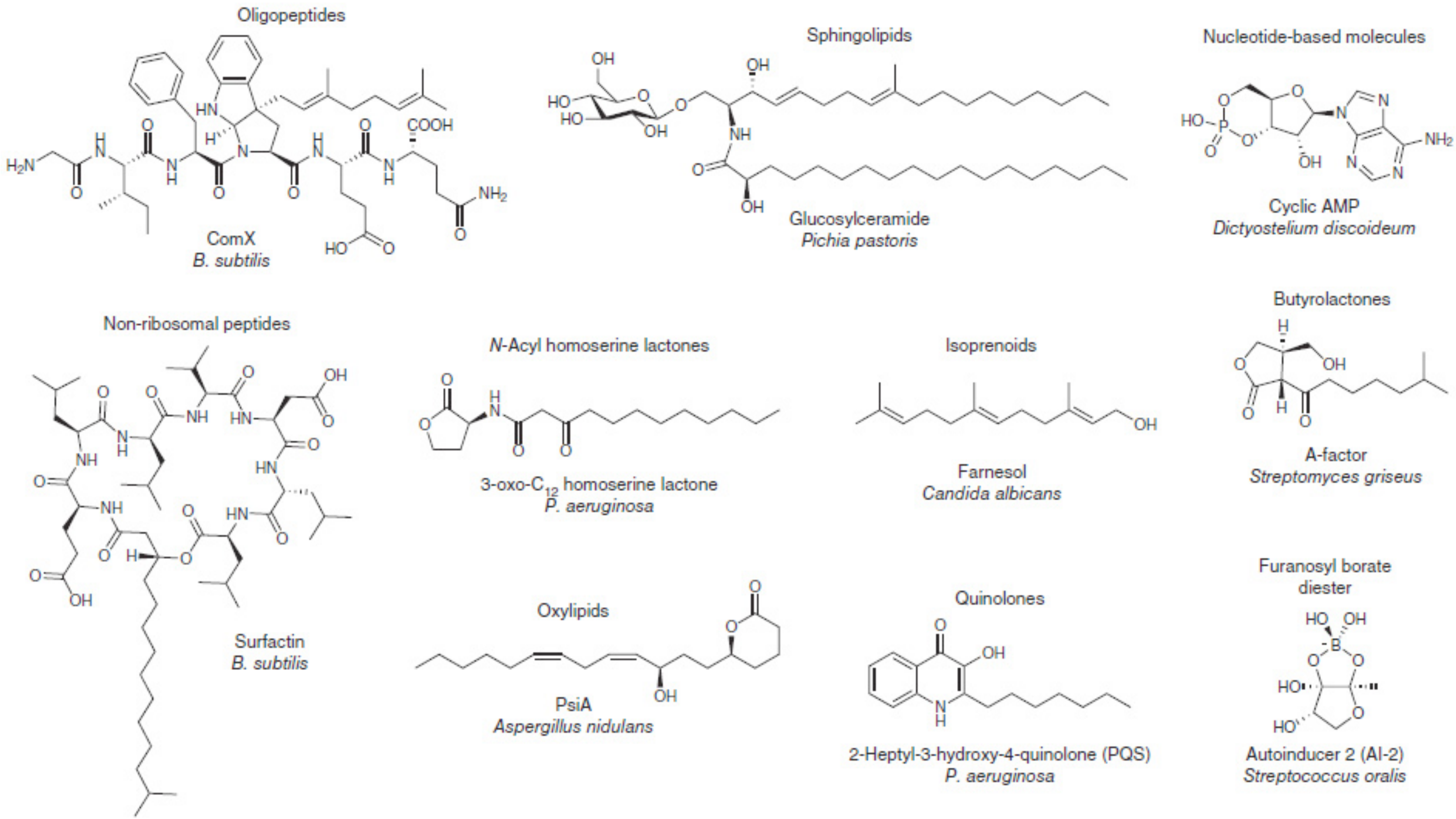
(d)

Stigmatella aurantiaca
Caroline Williams
New Scientist 16 July 2011


Quorum Sensing
HSL secreted
Relatively low HSL concentration
PLANKTONIC
Modified metabolism
Locally high HSL concentration
HSL secreted
HSL receptors
BIOFILM


AHLs
LuxR
LuxR
Lux I
Target genes

Chemical diversity of quorum-sensing molecules



Microbial metabolic exchange—the chemotype-to-phenotype link

Vanessa V Phelan, Wei-Ting Liu, Kit Pogliano & Pieter C Dorrestein

Nature Chemical Biology **8**, 26–35 (2012) doi:10.1038/nchembio.739

nature Vol 463|21 January 2010|doi:10.1038/nature08753

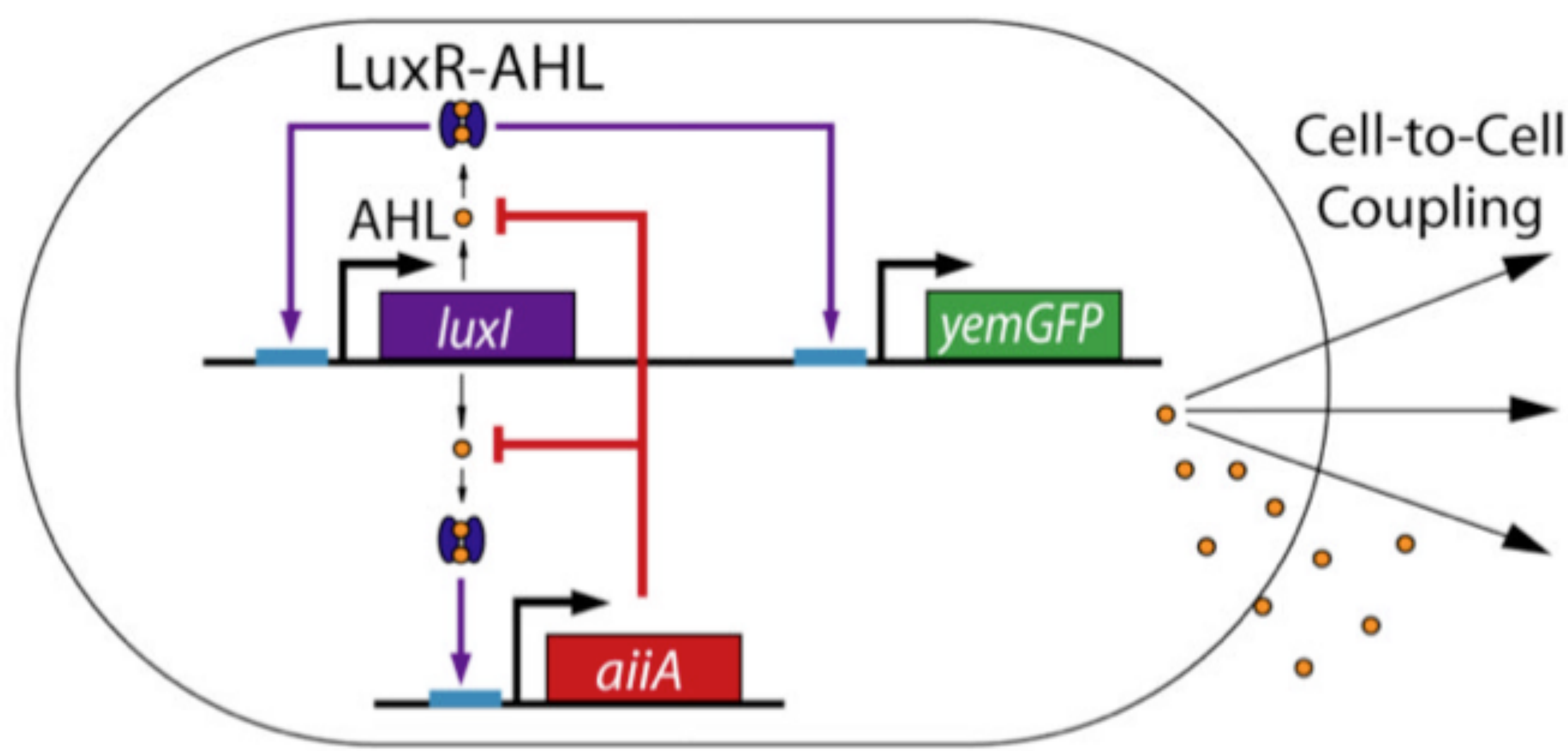
ARTICLES

A synchronized quorum of genetic clocks

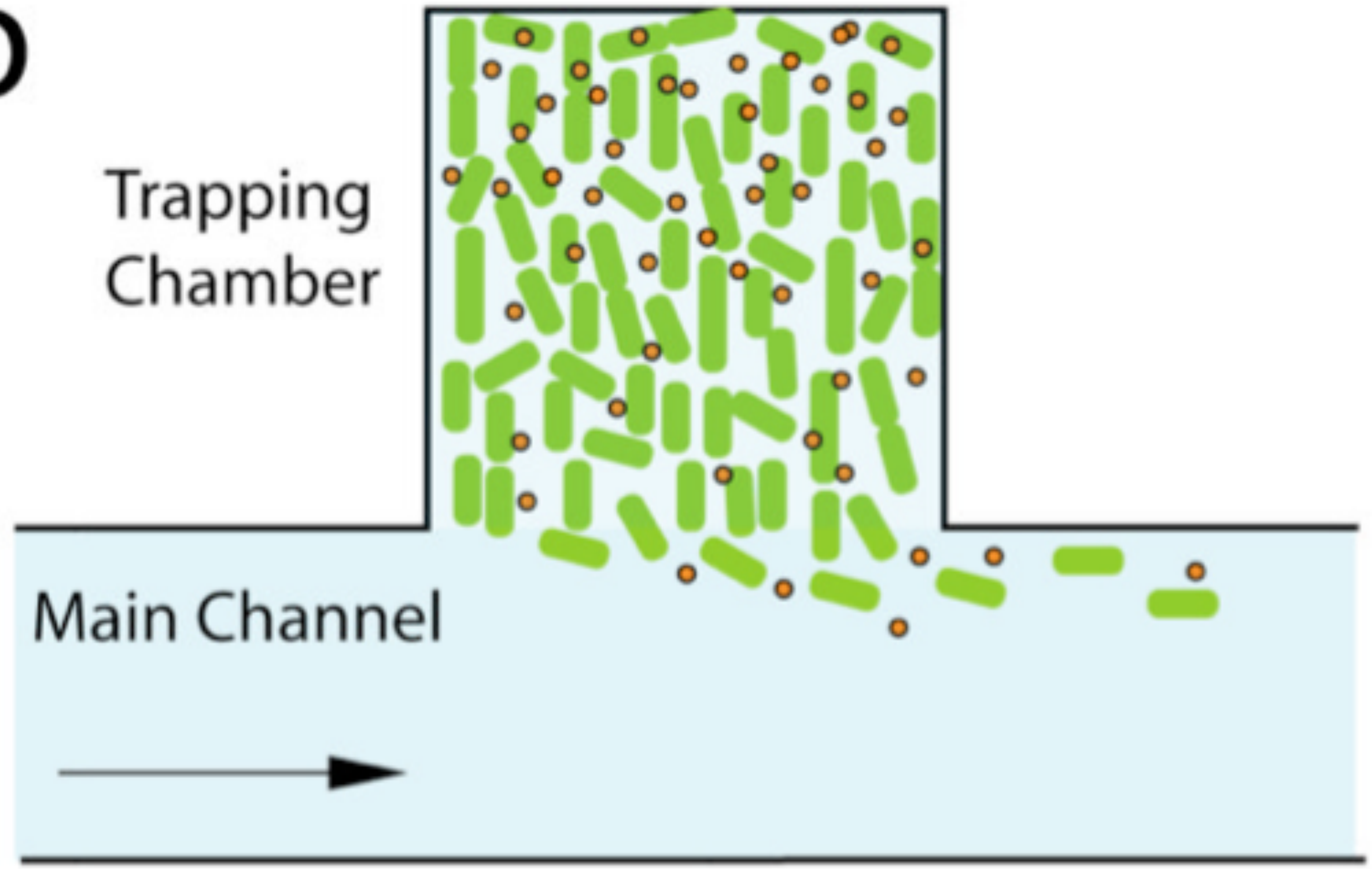
Tal Danino[1]*, Octavio Mondragón-Palomino[1]*, Lev Tsimring[2] & Jeff Hasty[1,2,3]

The engineering of genetic circuits with predictive functionality in living cells represents a defining focus of the expanding field of synthetic biology. This focus was elegantly set in motion a decade ago with the design and construction of a genetic toggle switch and an oscillator, with subsequent highlights that have included circuits capable of pattern generation, noise shaping, edge detection and event counting. Here we describe an engineered gene network with global intercellular coupling that is capable of generating synchronized oscillations in a growing population of cells. Using microfluidic devices tailored for cellular populations at differing length scales, we investigate the collective synchronization properties along with spatiotemporal waves occurring at millimetre scales. We use computational modelling to describe quantitatively the observed dependence of the period and amplitude of the bulk oscillations on the flow rate. The synchronized genetic clock sets the stage for the use of microbes in the creation of a macroscopic biosensor with an oscillatory output. Furthermore, it provides a specific model system for the generation of a mechanistic description of emergent coordinated behaviour at the colony level.

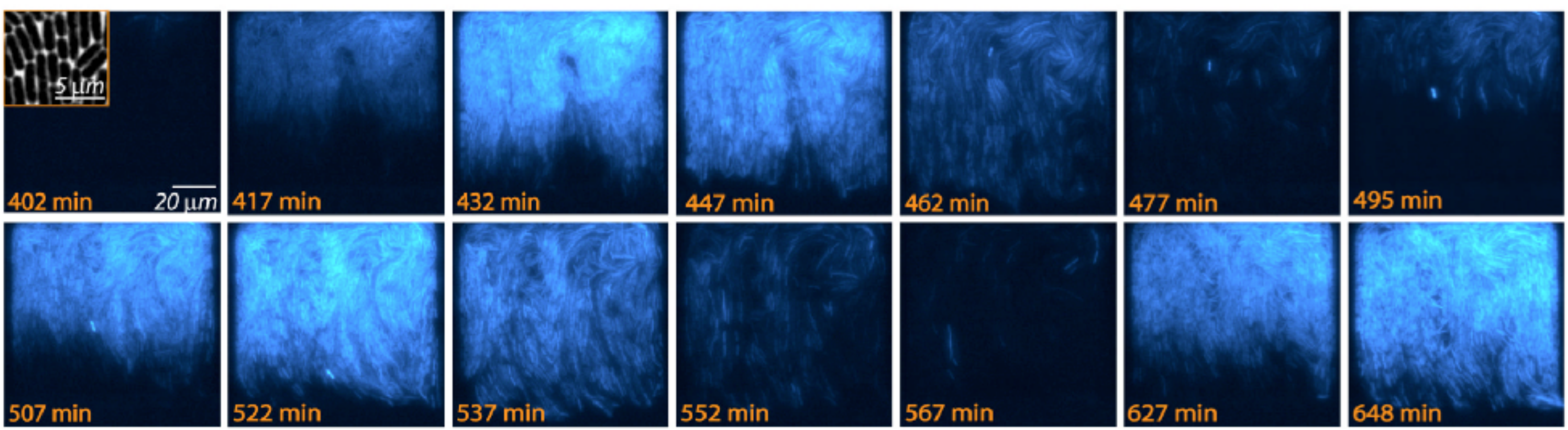
a

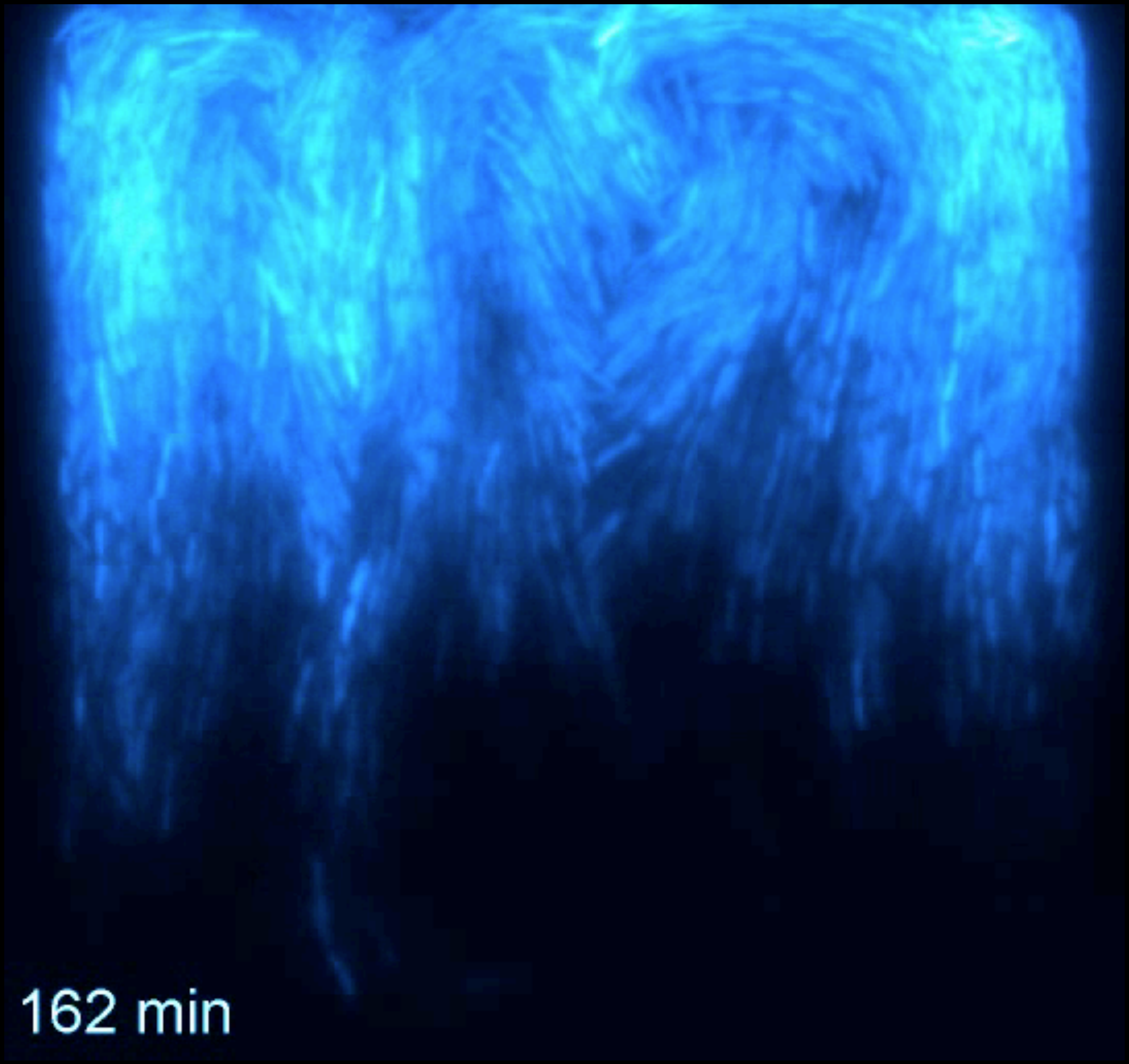


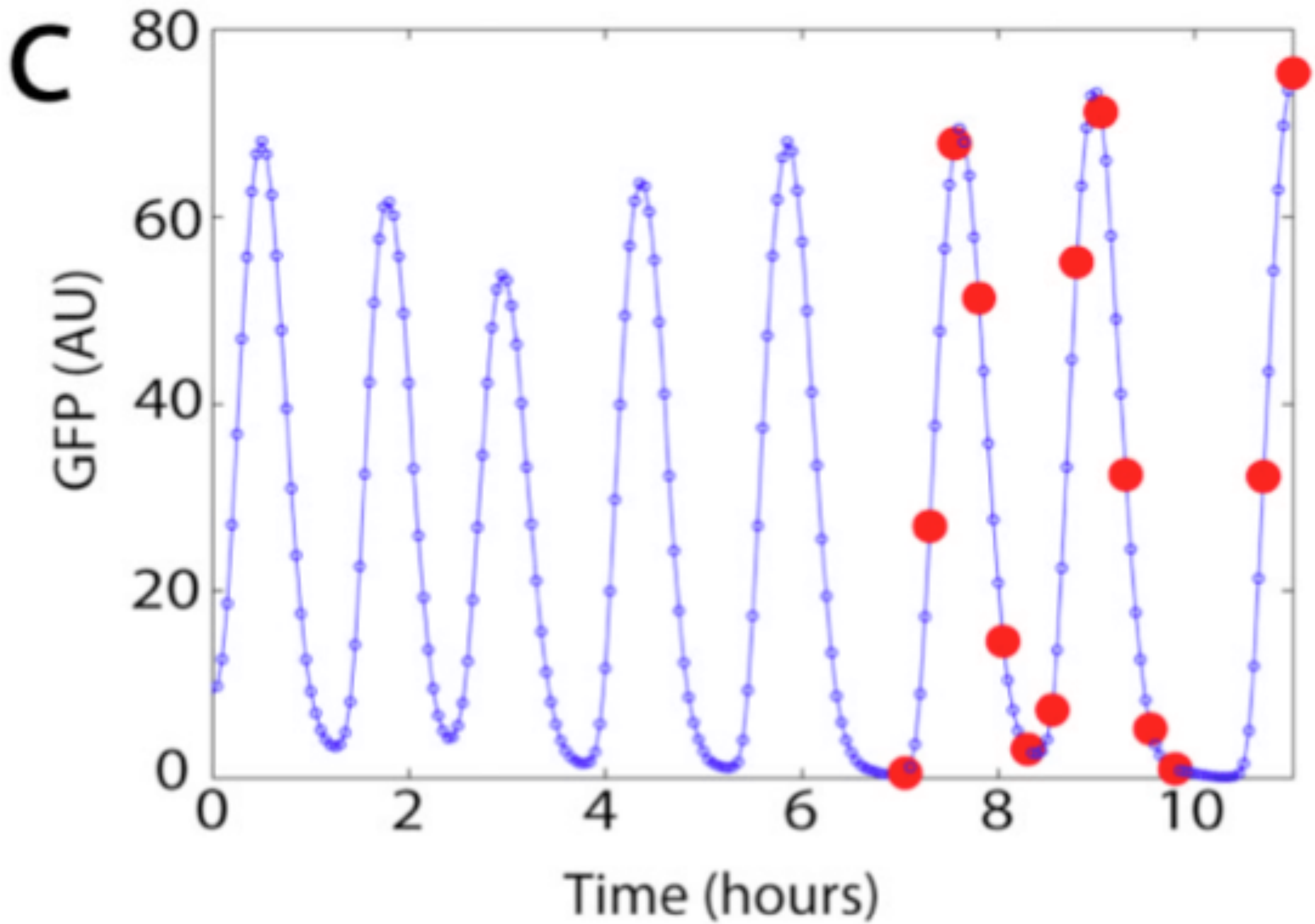
b


Trapping Chamber
Main Channel

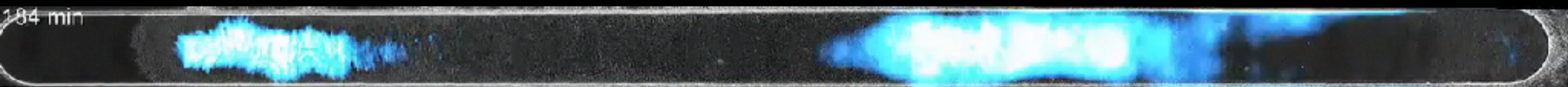
d

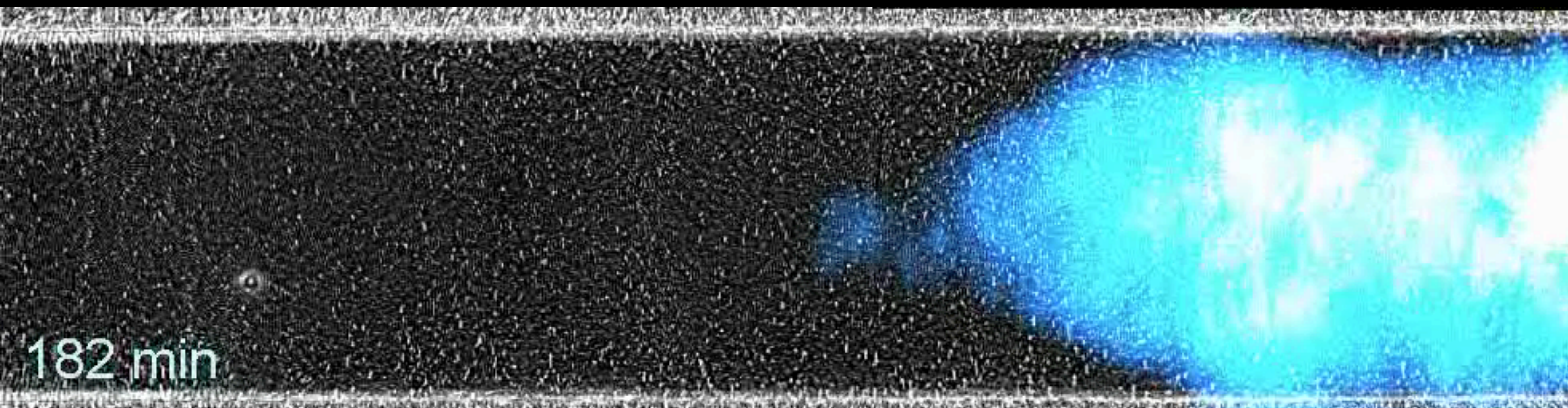

5 μm
20 μm
402 min
417 min
432 min
447 min
462 min
477 min
495 min
507 min
522 min
537 min
552 min
567 min
627 min
648 min

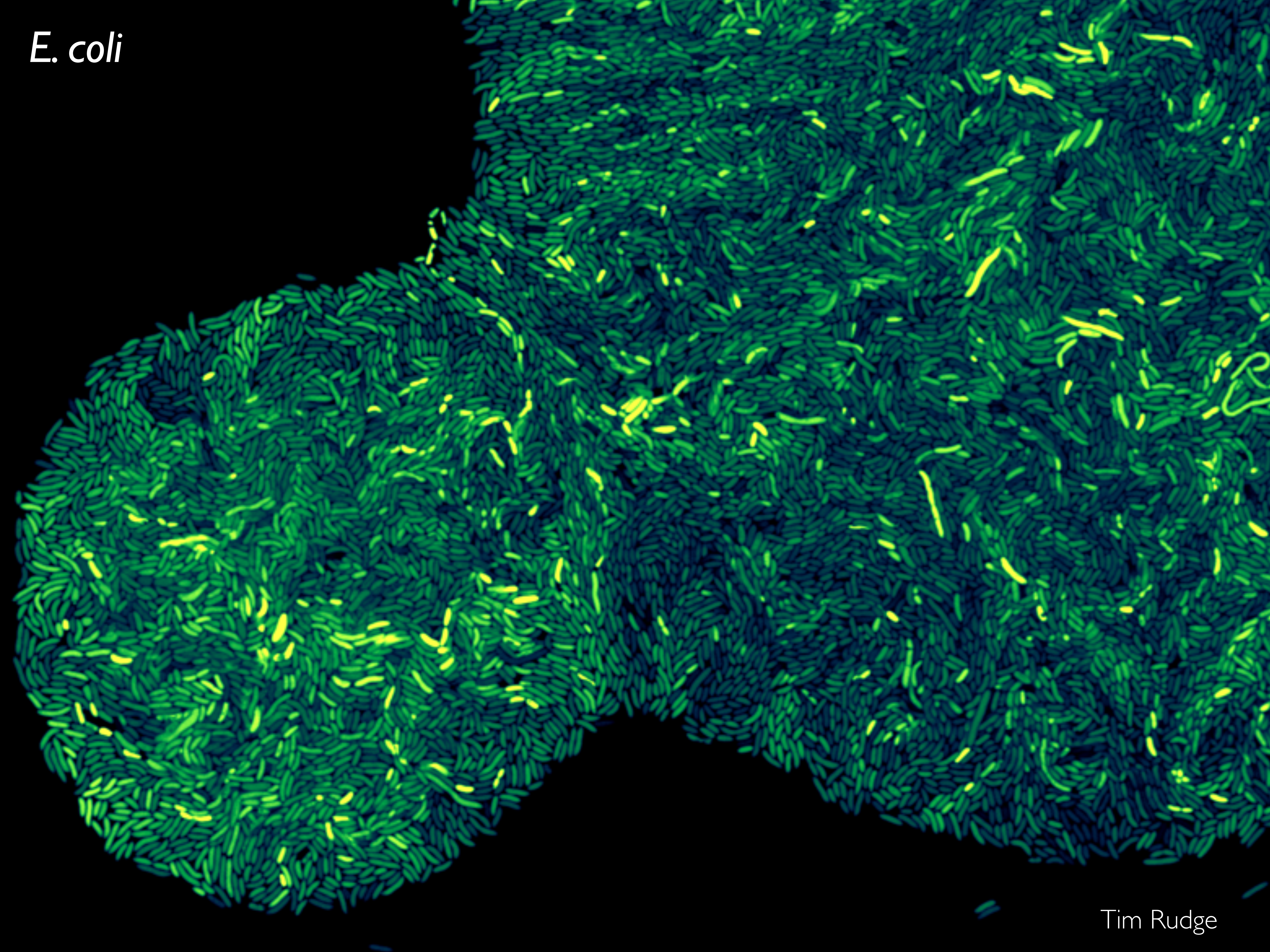

162 min


C
80
60
40
20
0
GFP (AU)
0
2
4
6
8
10
Time (hours)

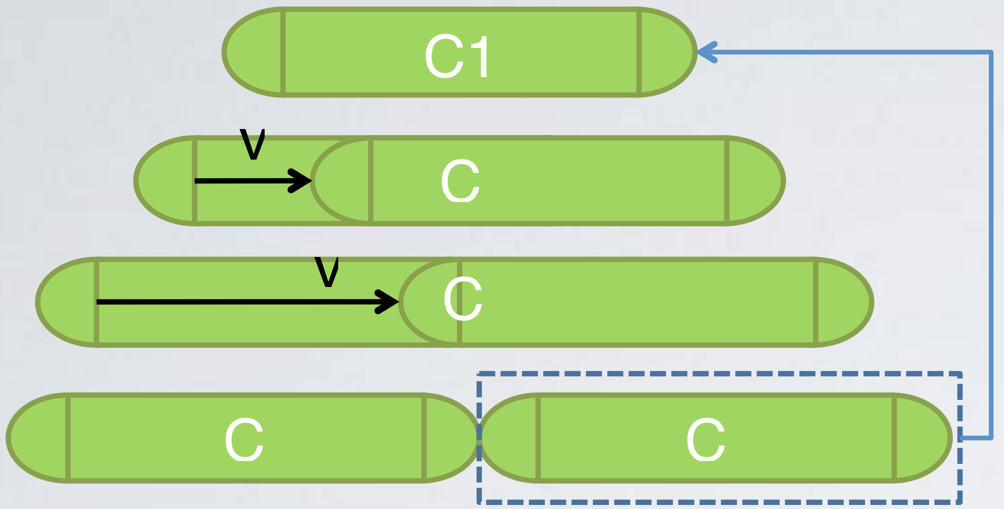

288 min


184 min

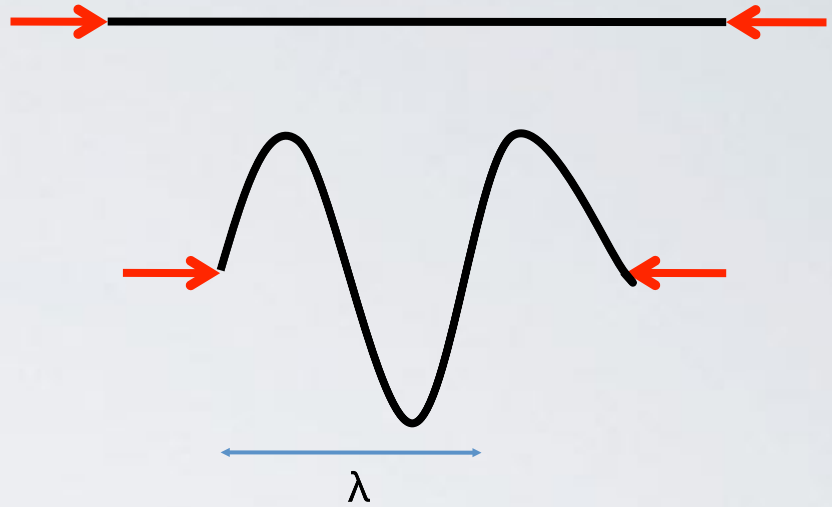

182 min

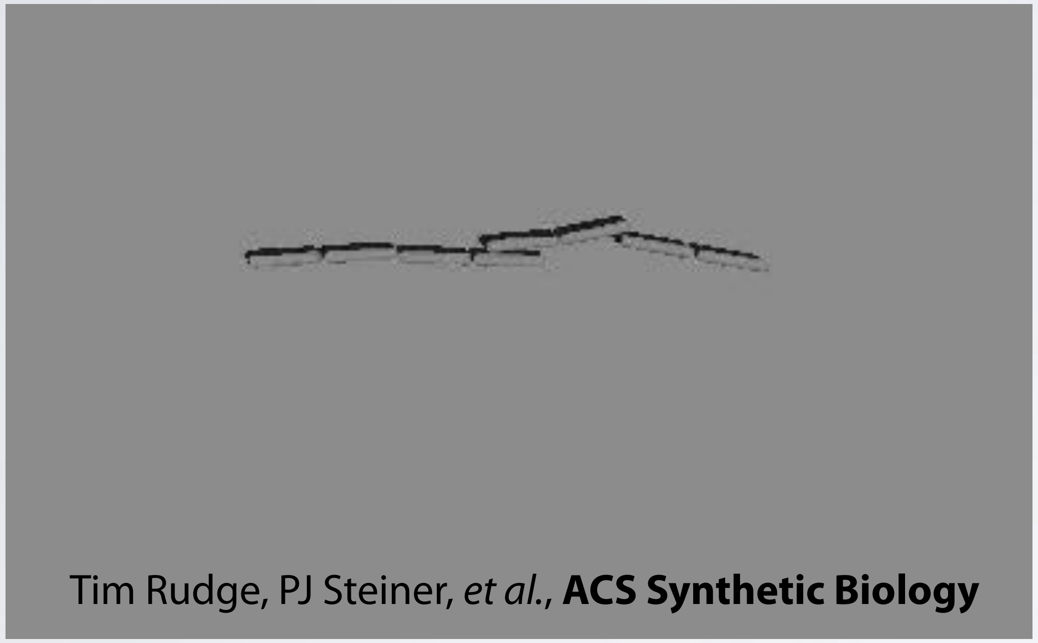

E. coli
Tim Rudge

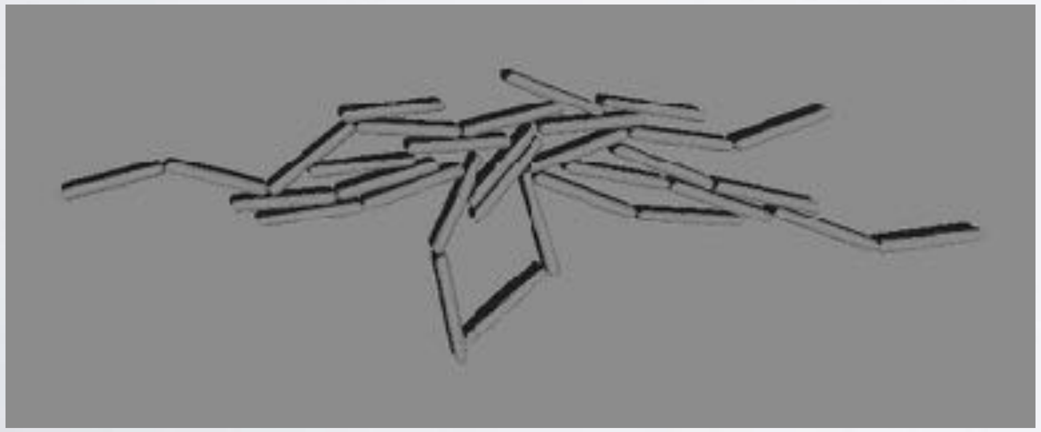
3D Rigid body dynamics

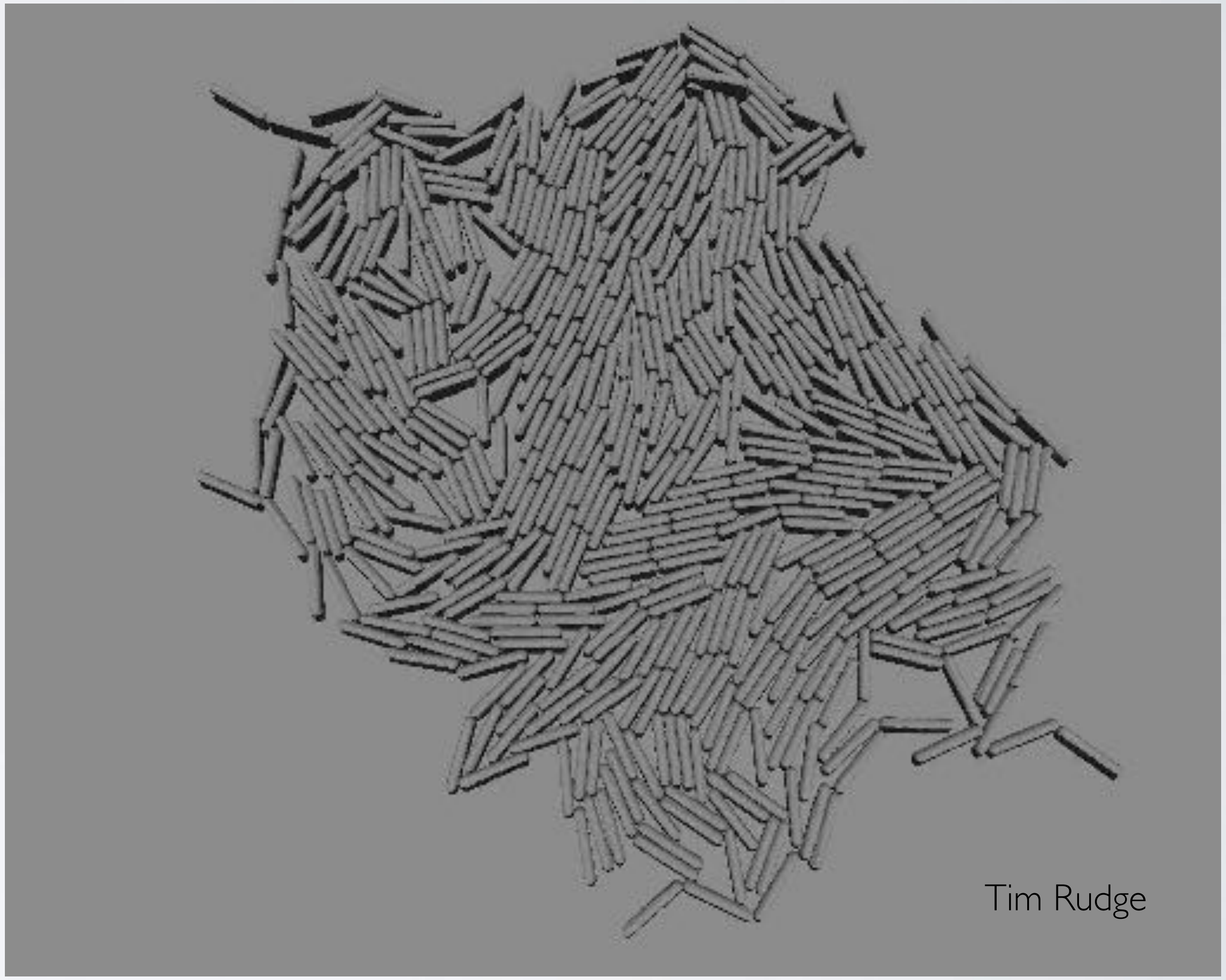


Folding growth pattern due to buckling





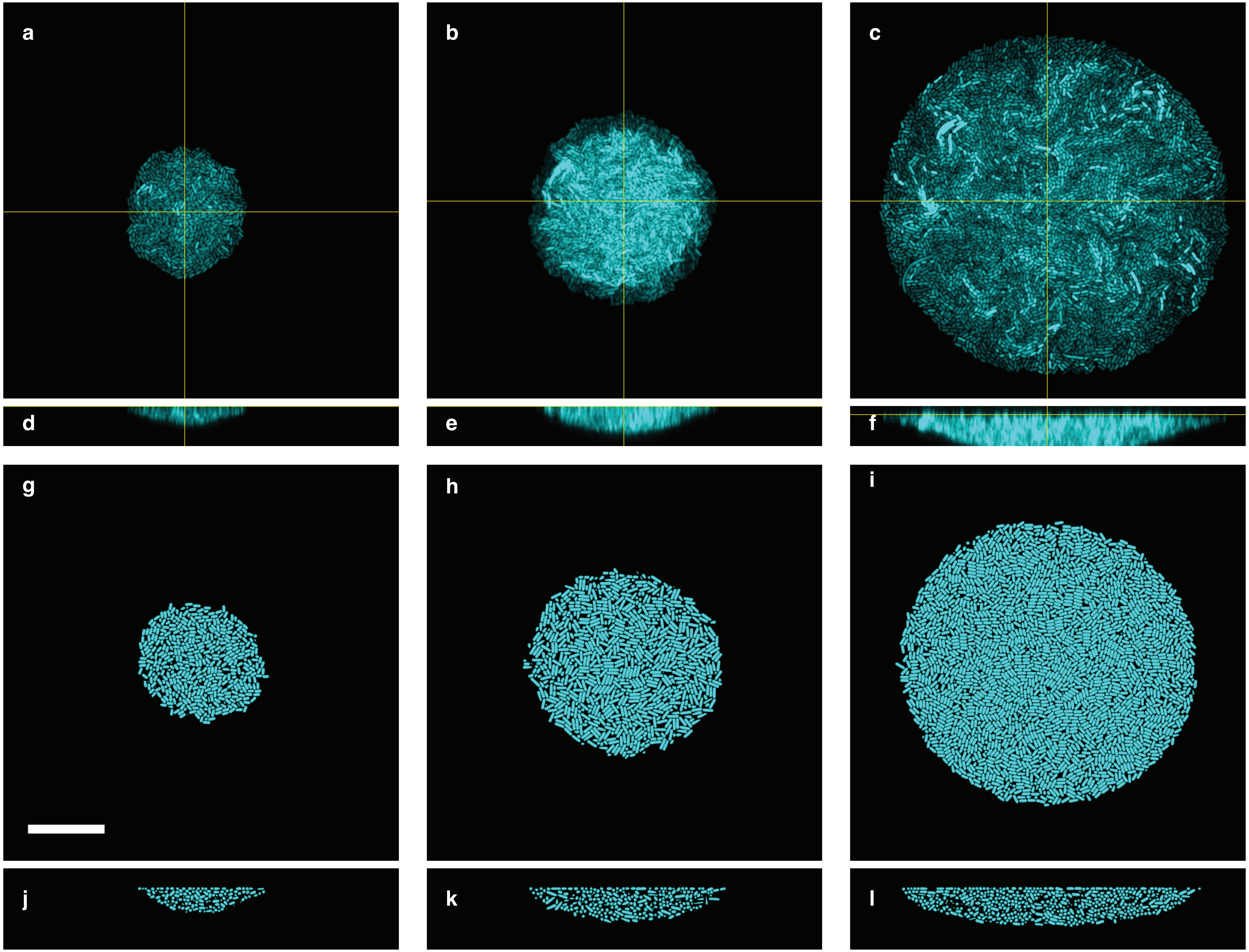




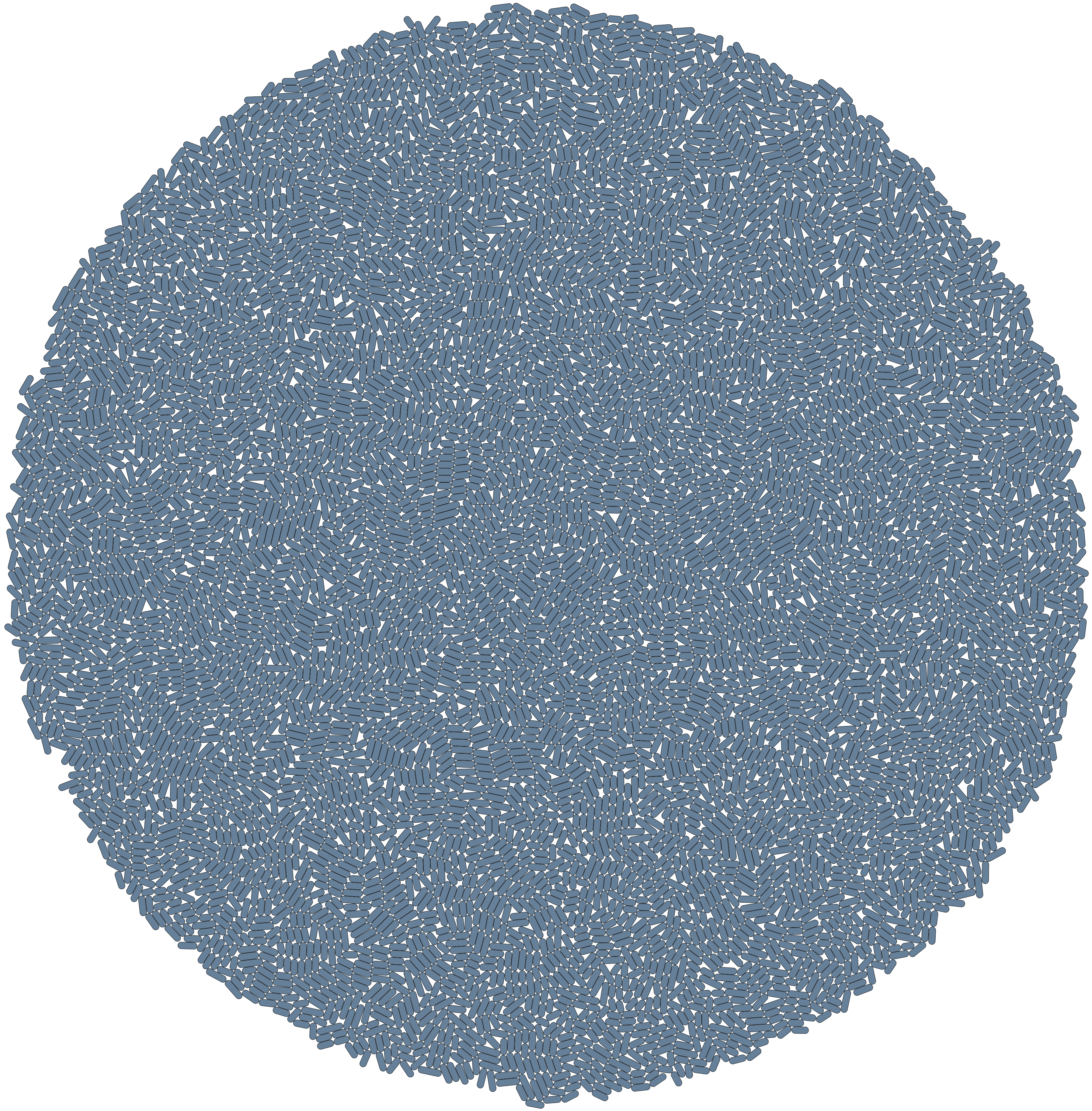
Modelling of bacterial cell growth

GPU accelerated models for biofilm growth

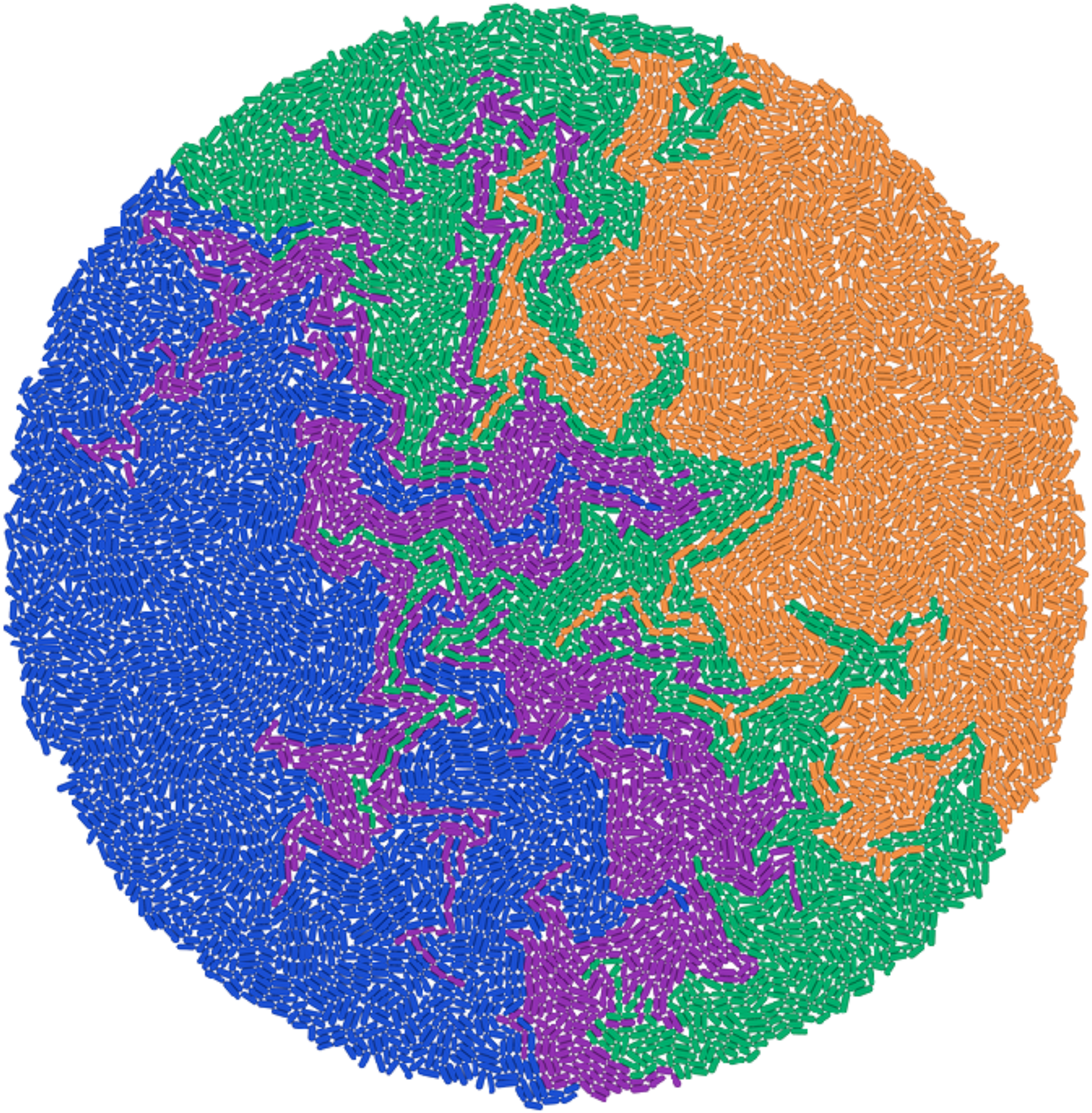
Video game graphics technology - gives interactive visualisation up to >100,000 cells



Tim Rudge, PJ Steiner, *et al.*, **ACS Synthetic Biology**

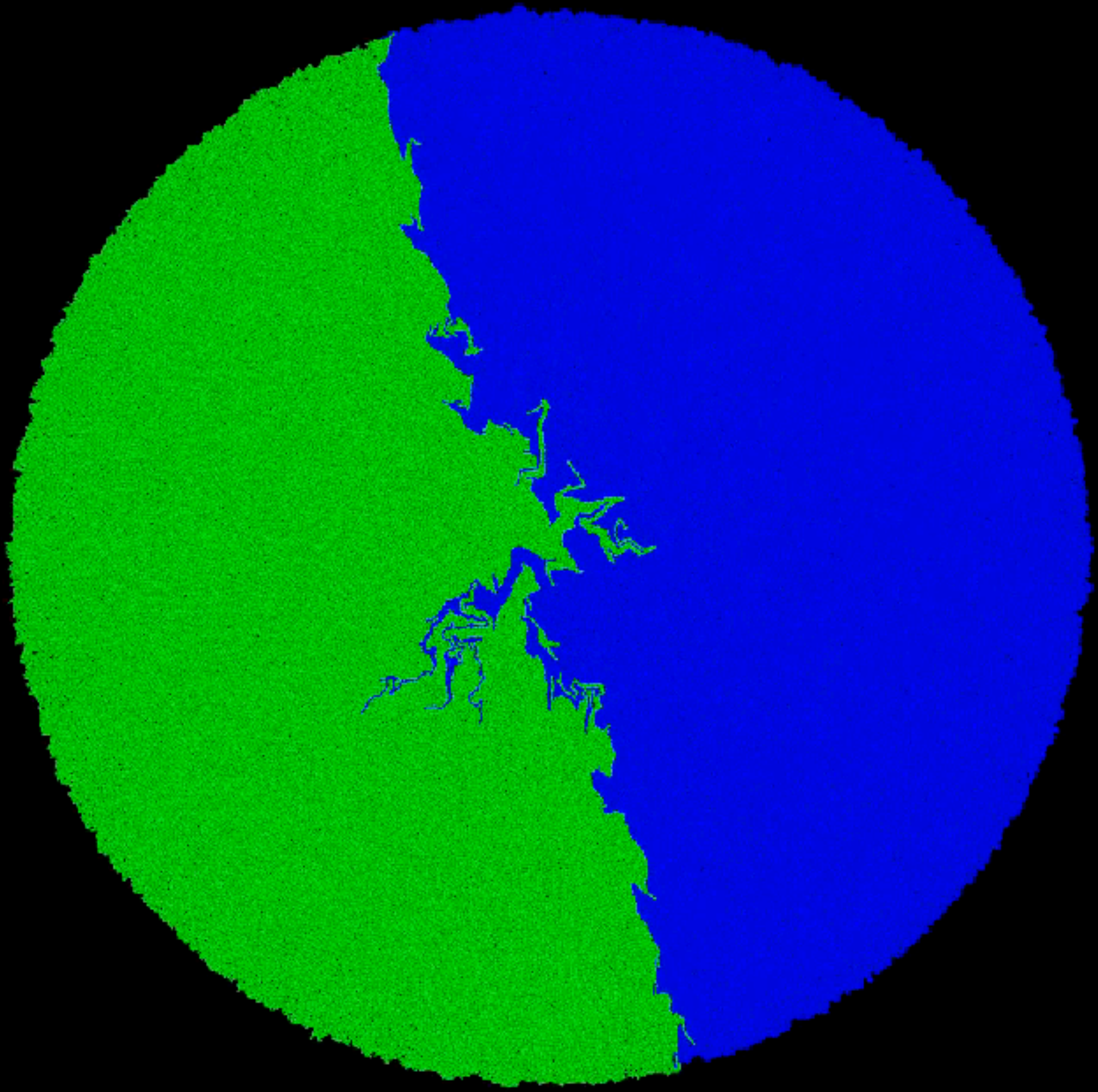


Tim Rudge and PJ Steiner



Tim Rudge and PJ Steiner

CellModeller4

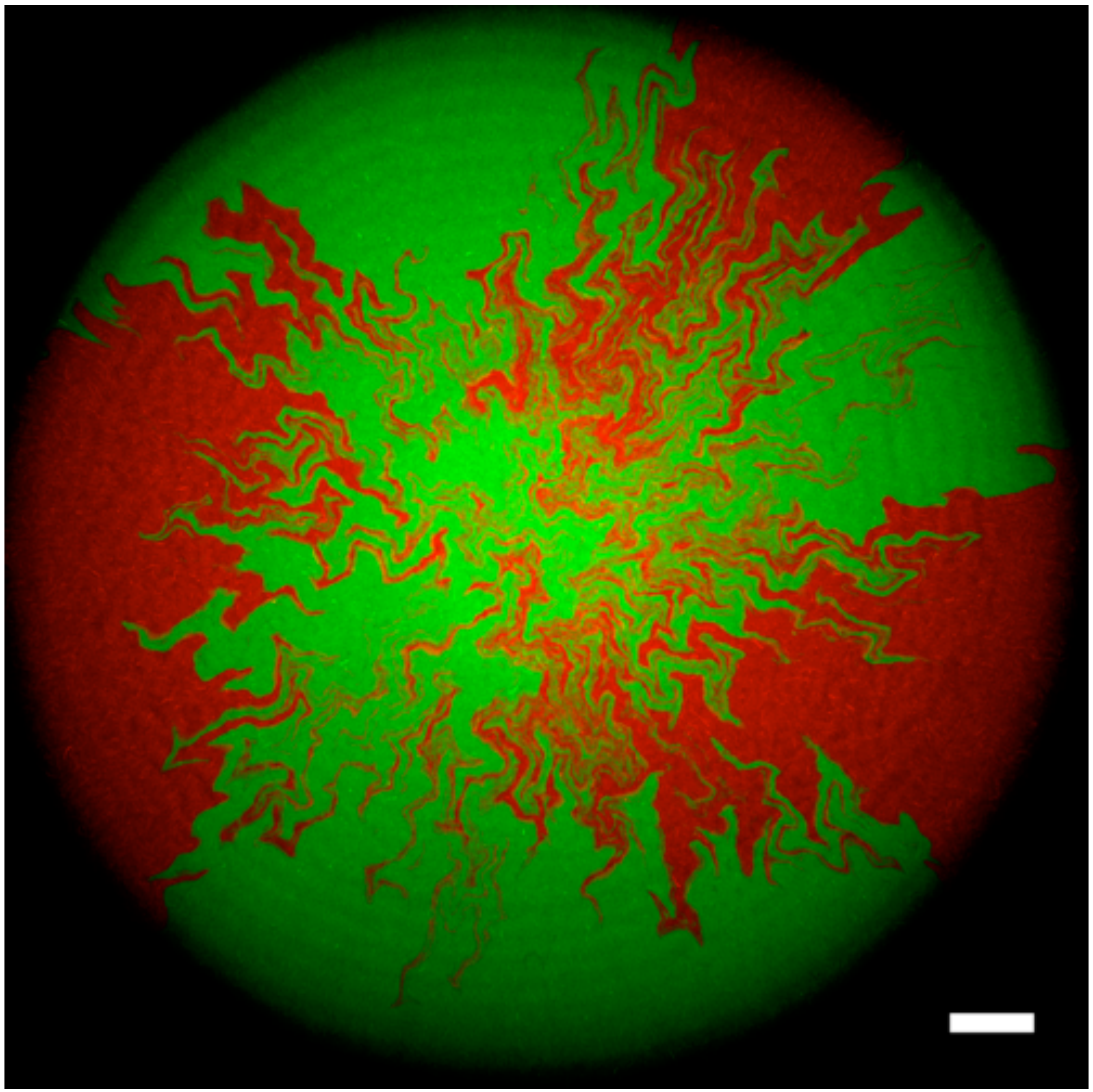


Growth of marked daughters into 100,000 cell colony

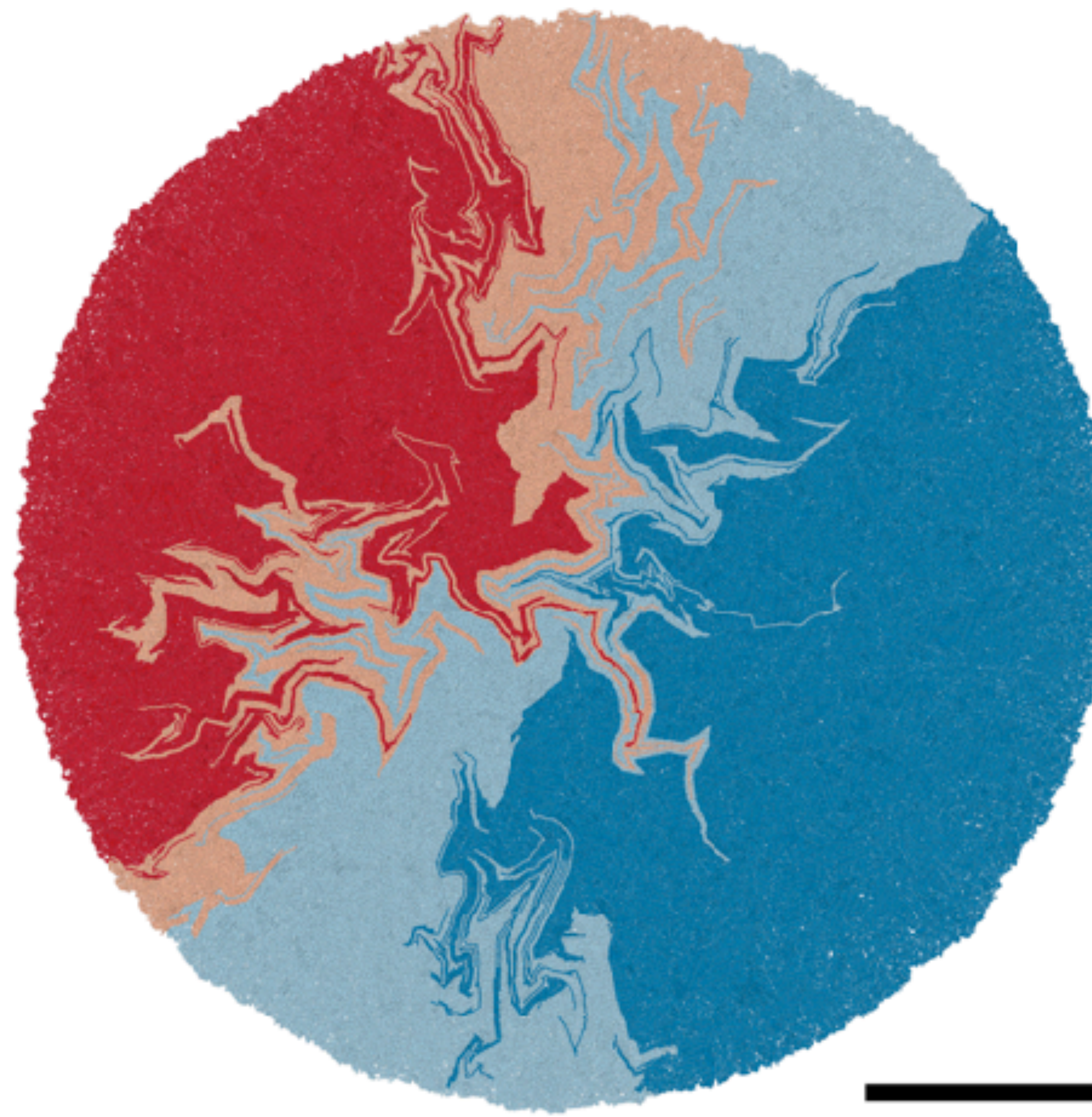
Tim Rudge & PJ Steiner

Colony comparison

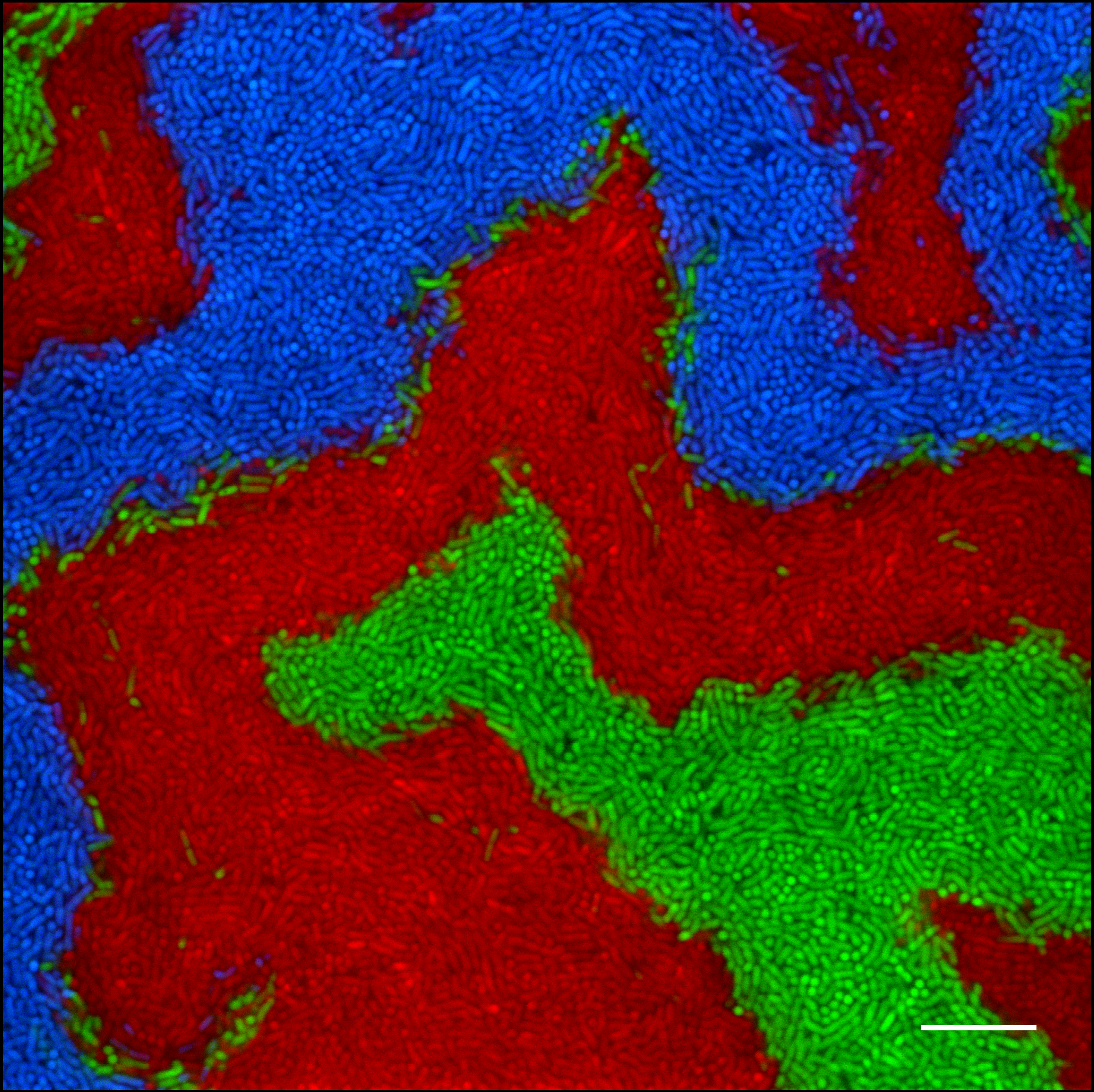
24 hour old *E. coli* colony, with marked lineages using a plasmid segregation technique



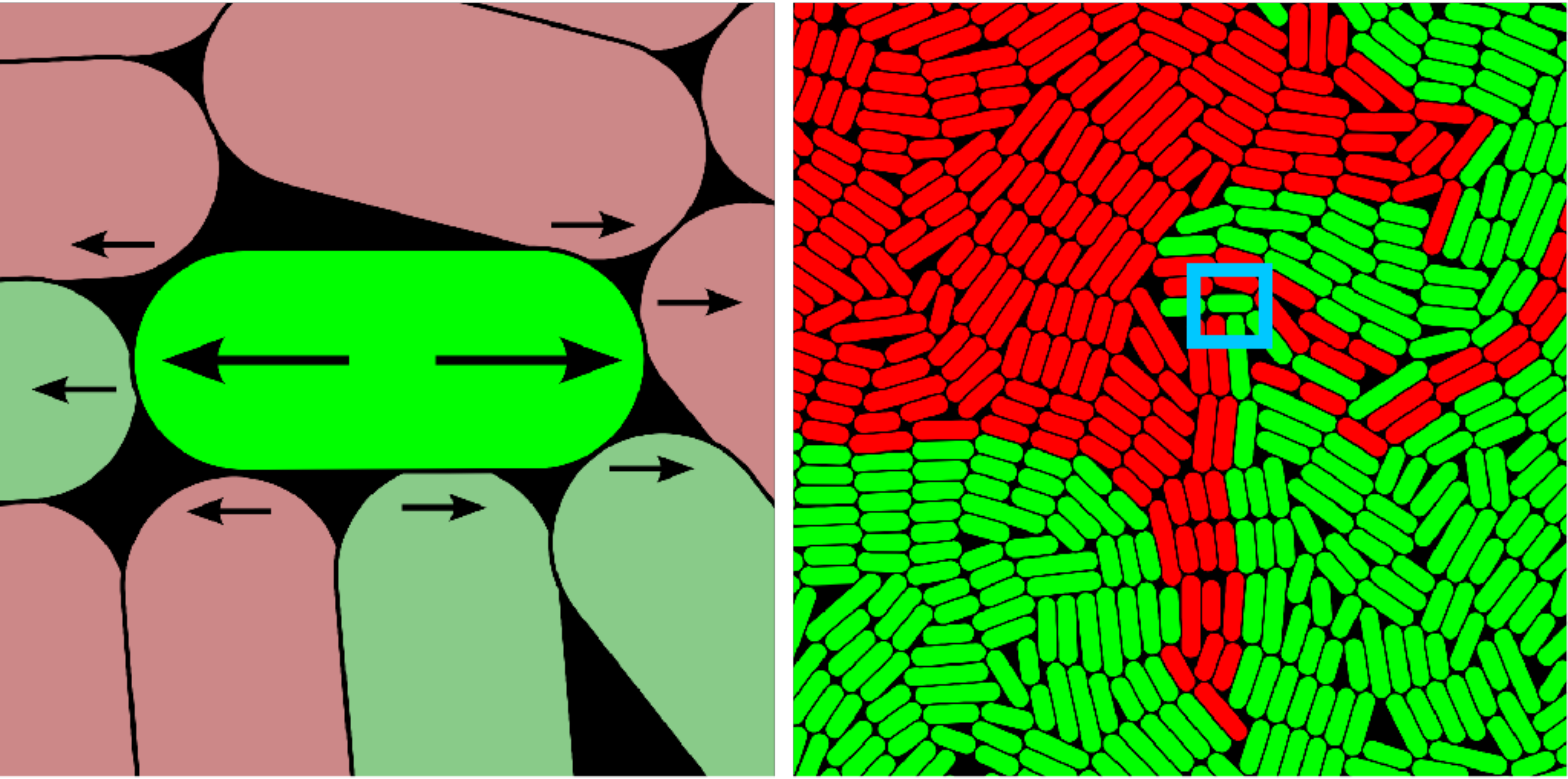
CellModeller colony with 100,000 cells, initiated by 4 adjacent cells

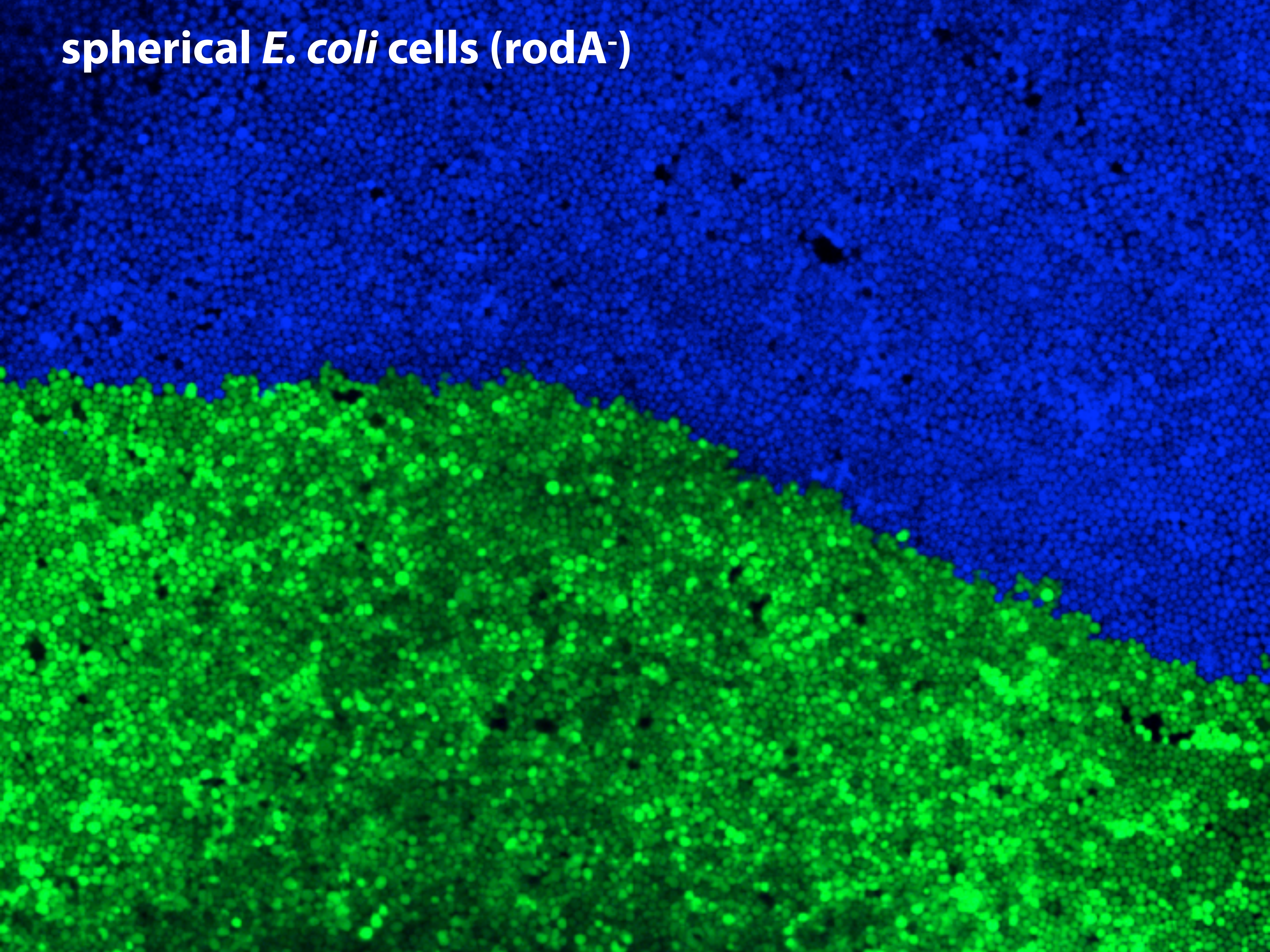


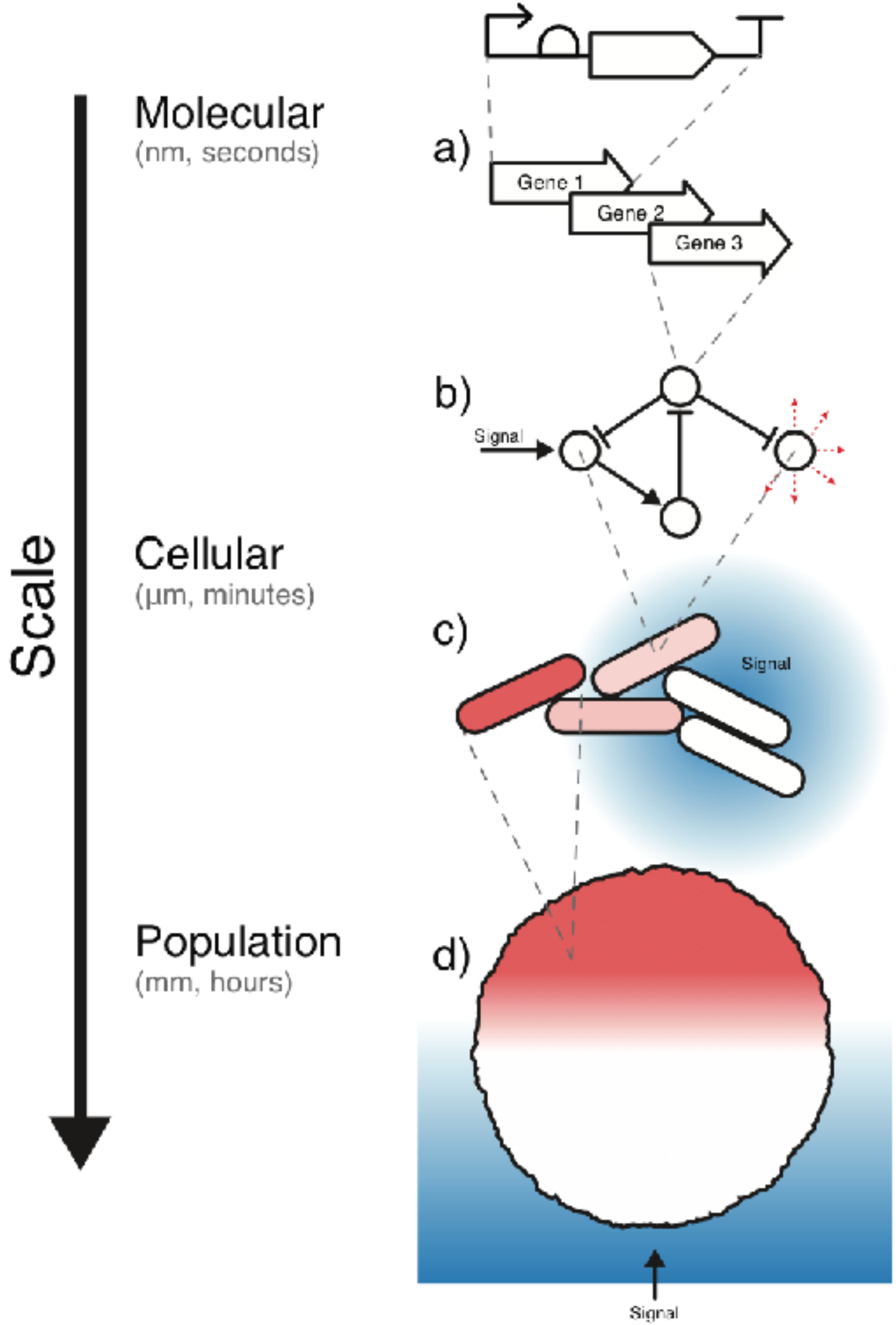
Scale is 100μm



Fernan Federici

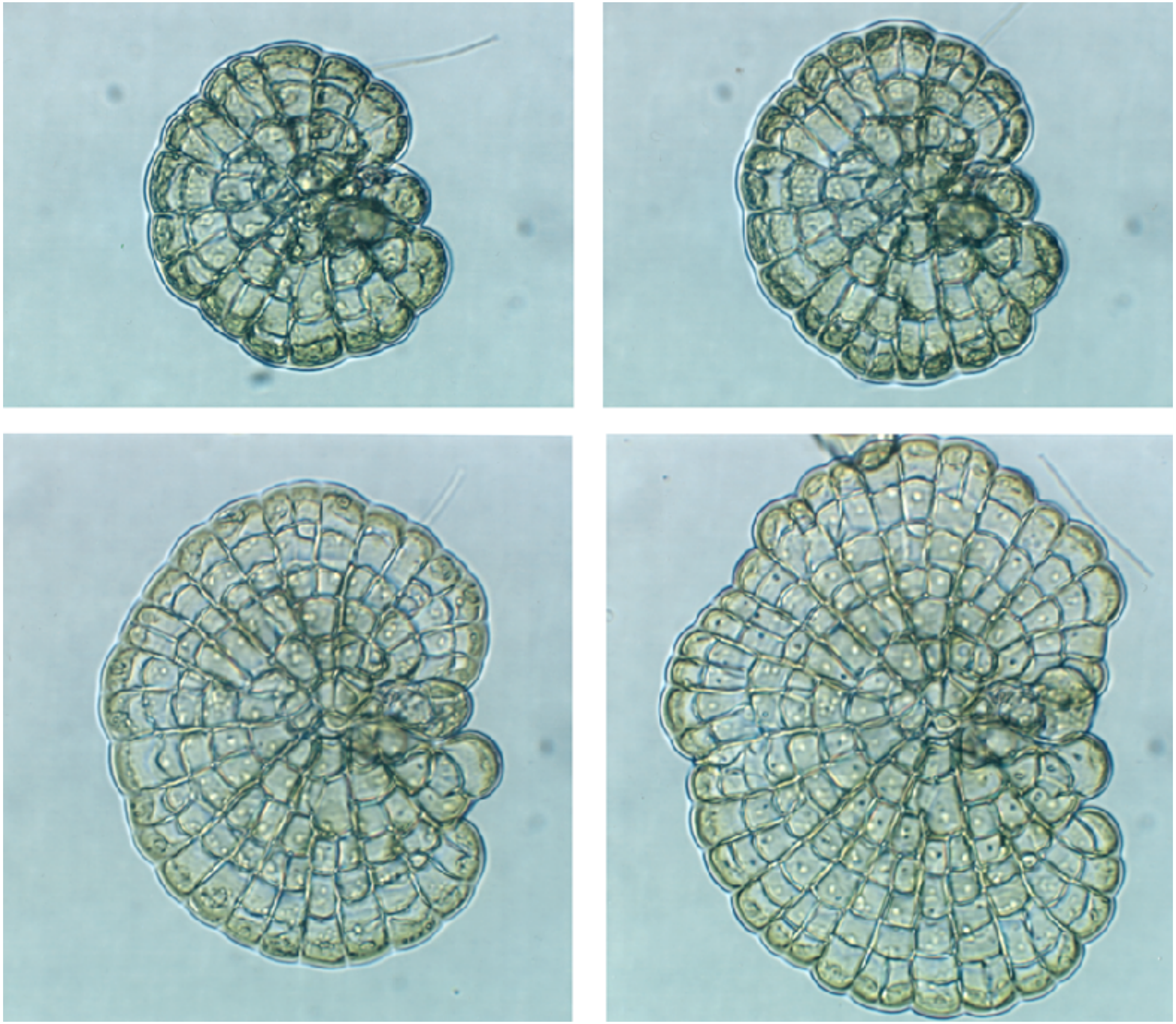



spherical *E. coli* cells (rodA⁻)



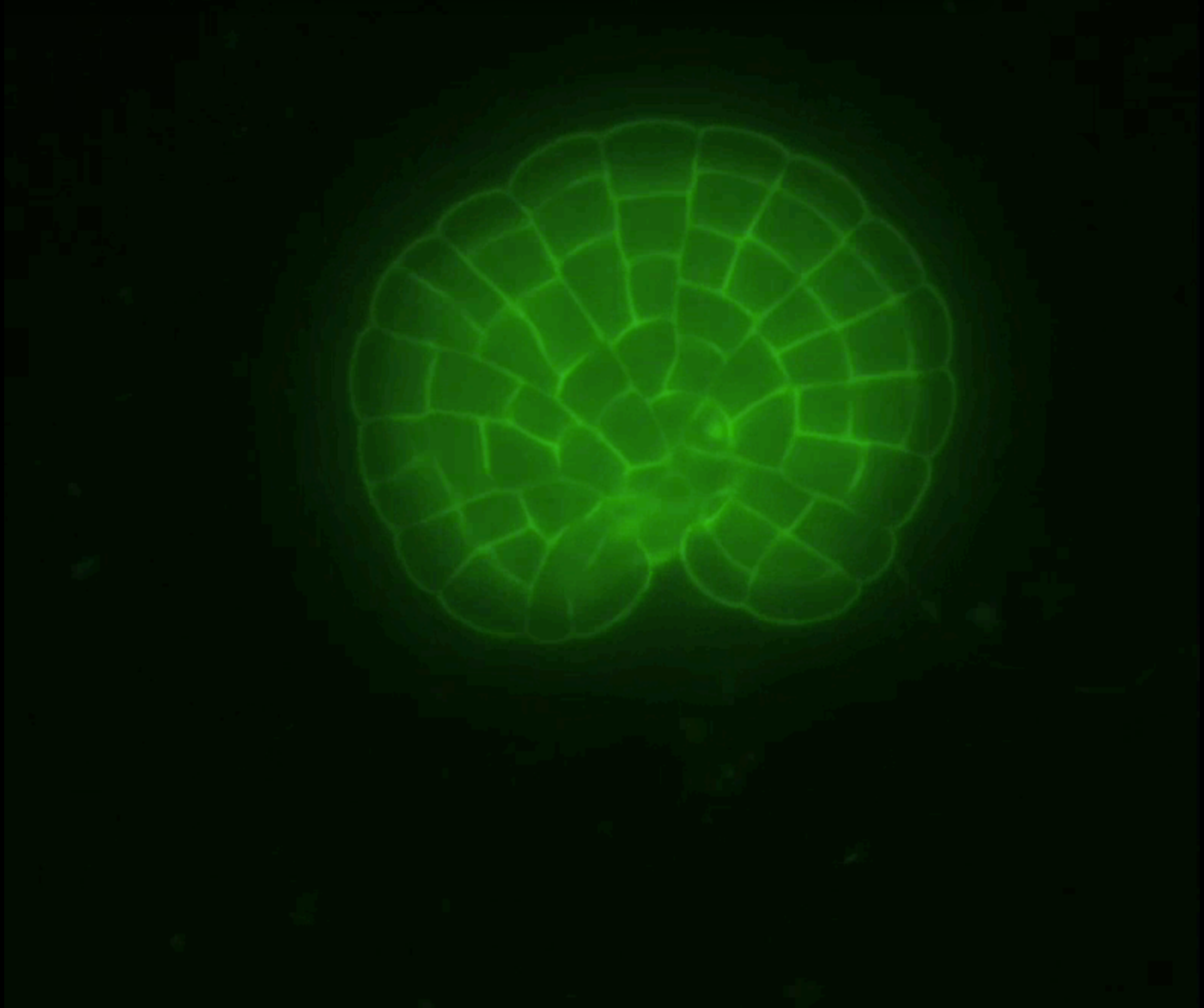
Scale
Molecular
(nm, seconds)
Cellular
(µm, minutes)
Population
(mm, hours)
a)
Gene 1
Gene 2
Gene 3
b)
Signal
c)
Signal
d)
Signal

Coleochaete: simplest "plant-like" systems

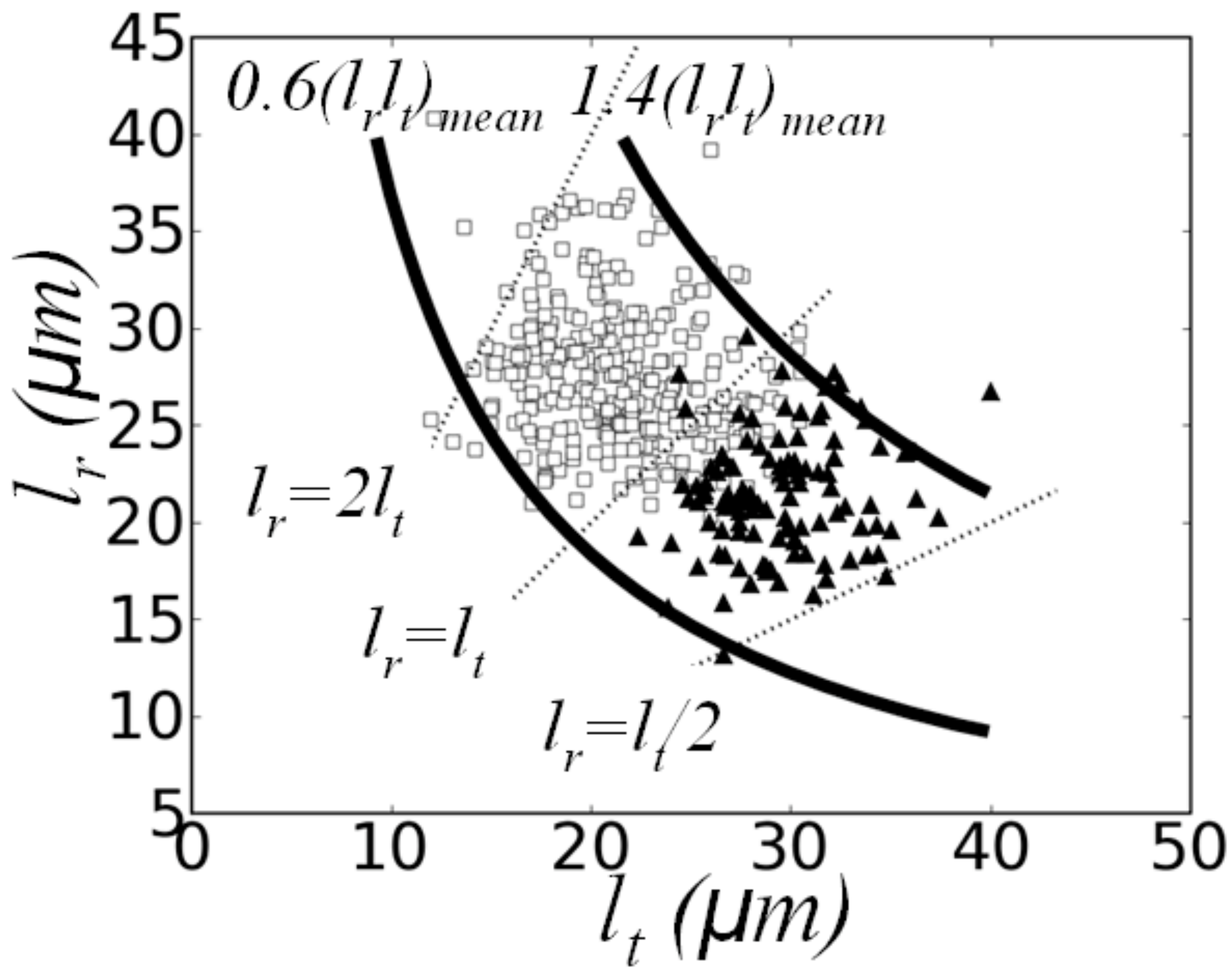


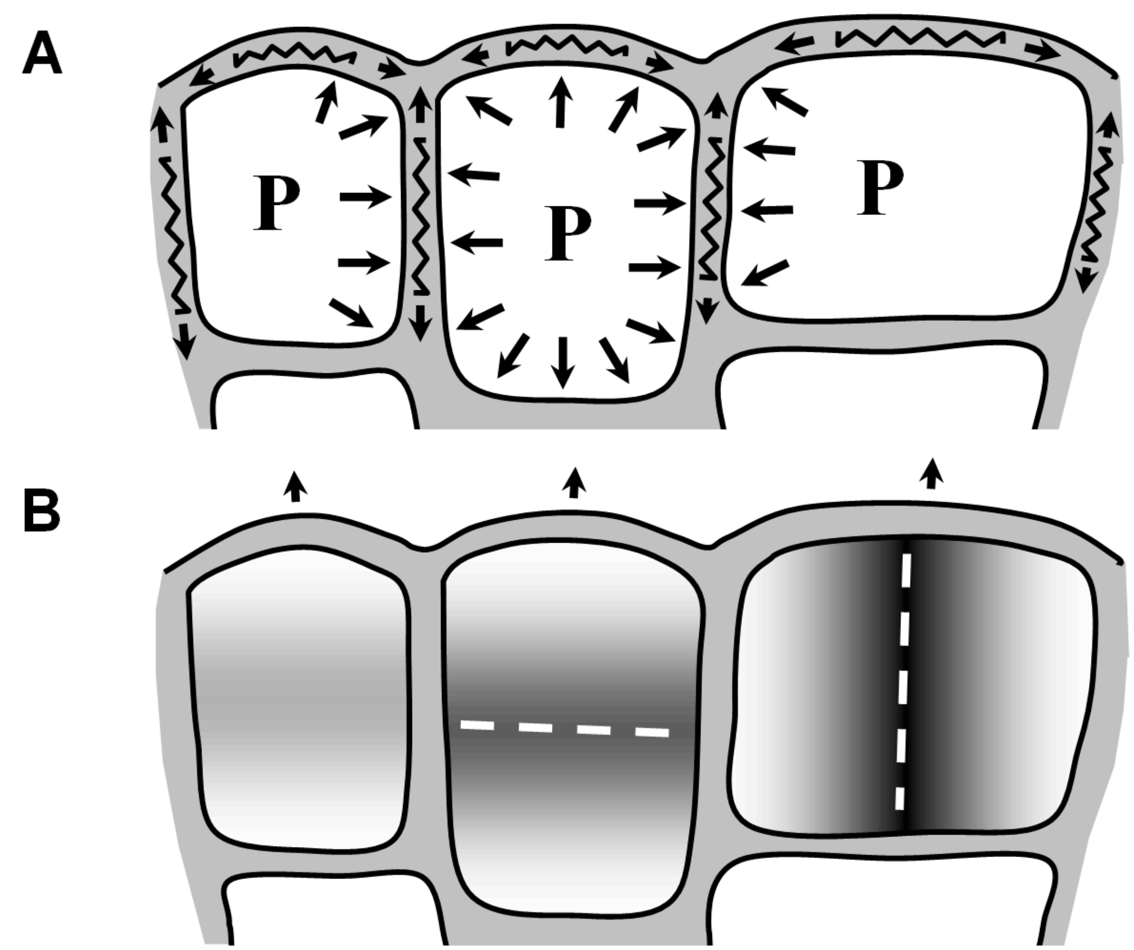
Time series of the growth of *Coleochaete orbicularis*

Simple meristem with cell division limited to a layer of cells at the circumference of the thallus



Rules for plant cell division

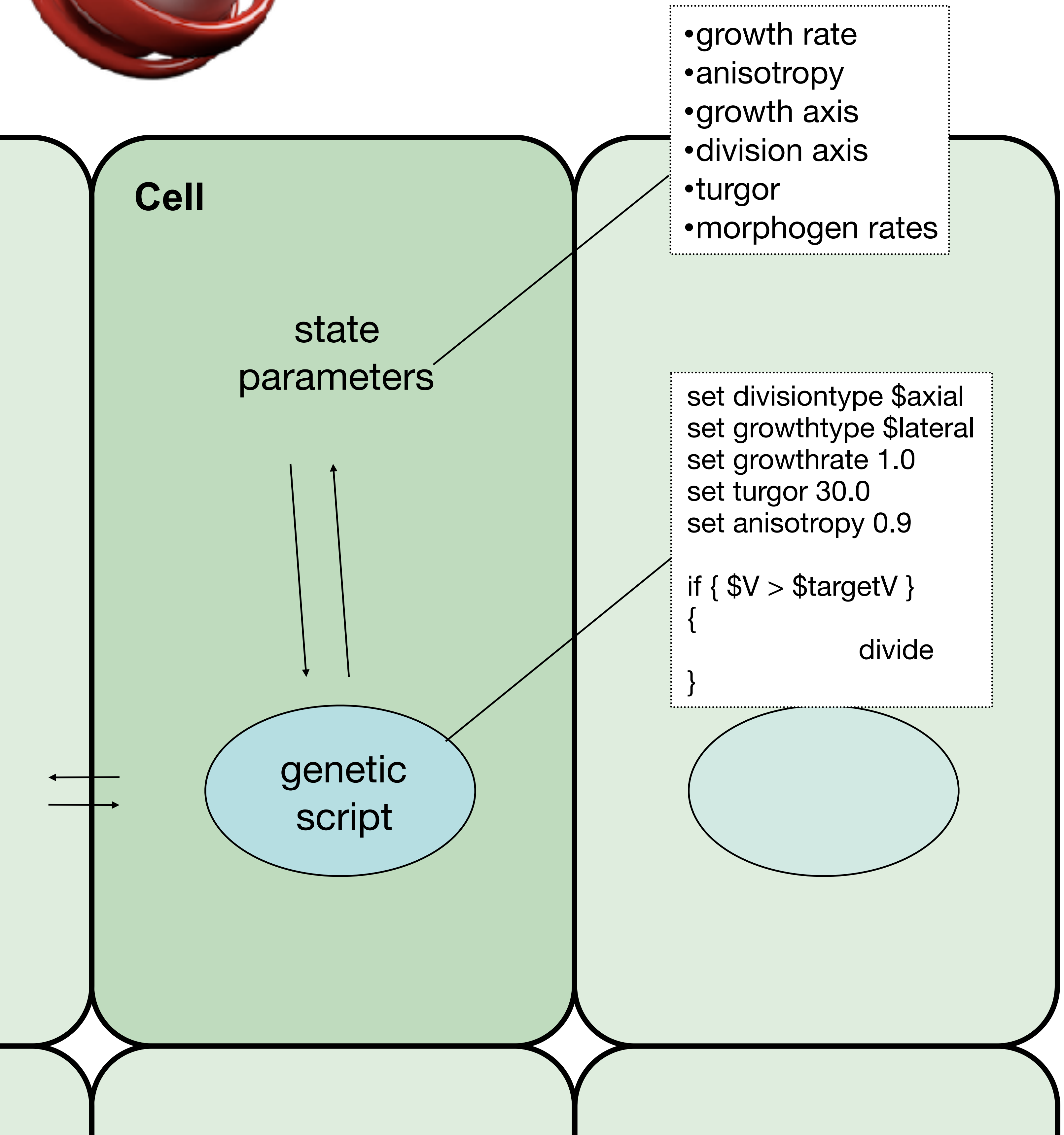


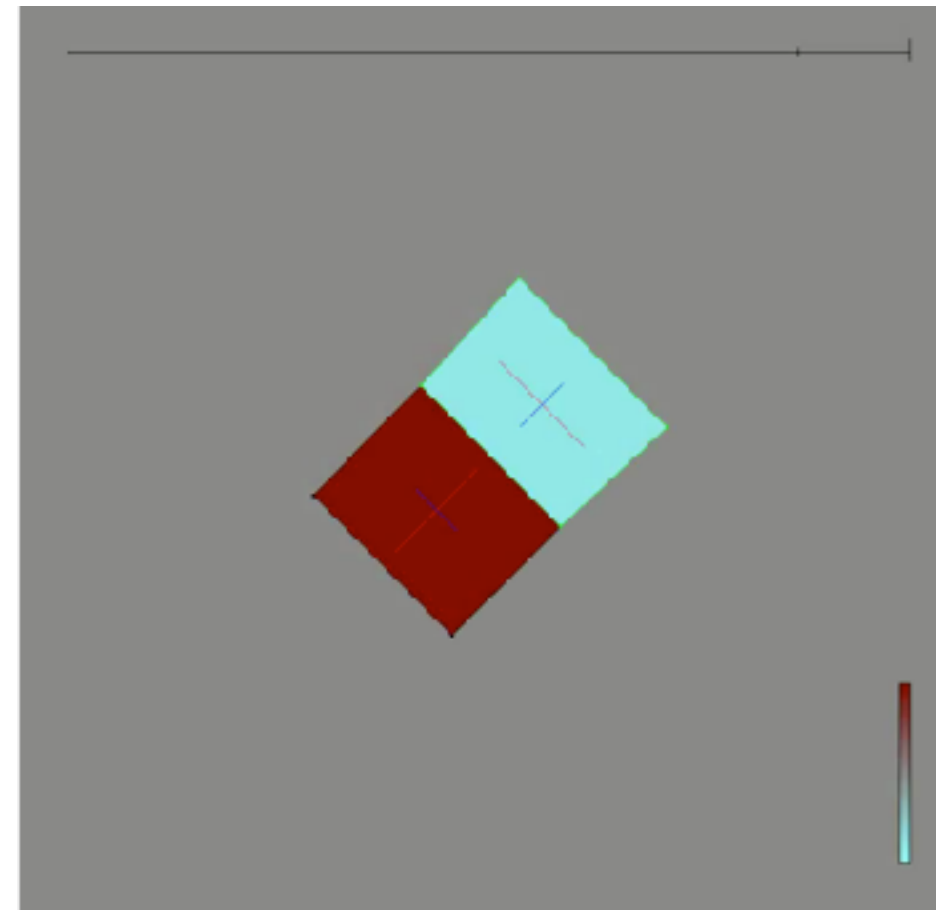


Coordination of plant cell division and expansion in a simple morphogenetic system

Lionel Dupuy, Jonathan Mackenzie, and Jim Haseloff, PNAS, 107:2711-2716, 2010.

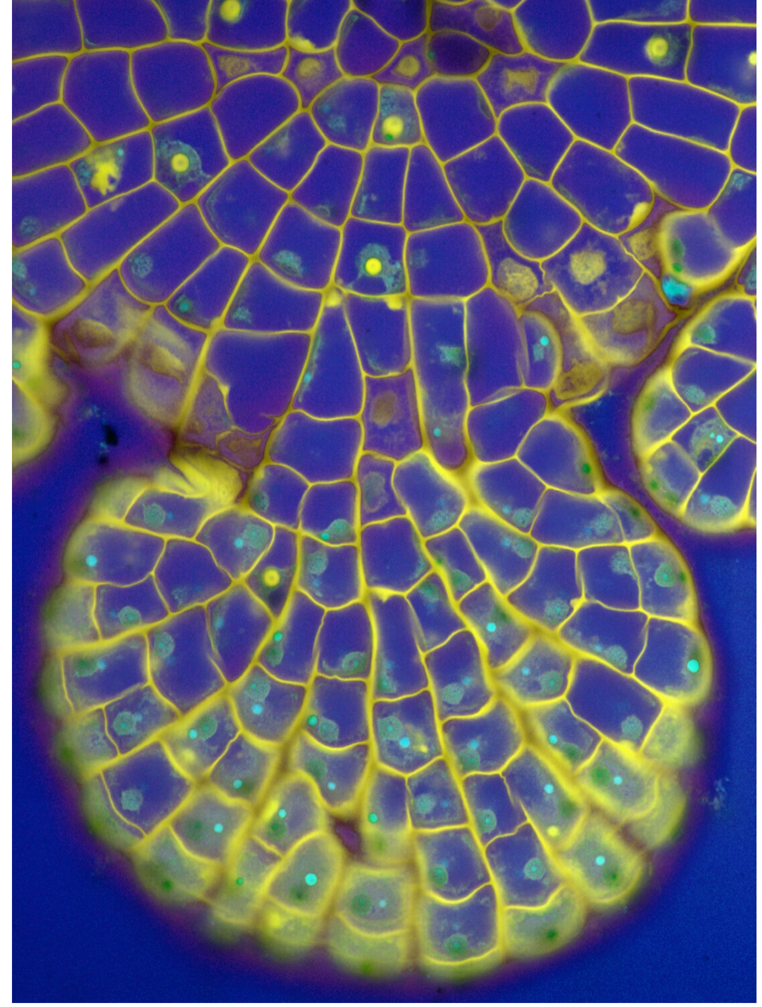
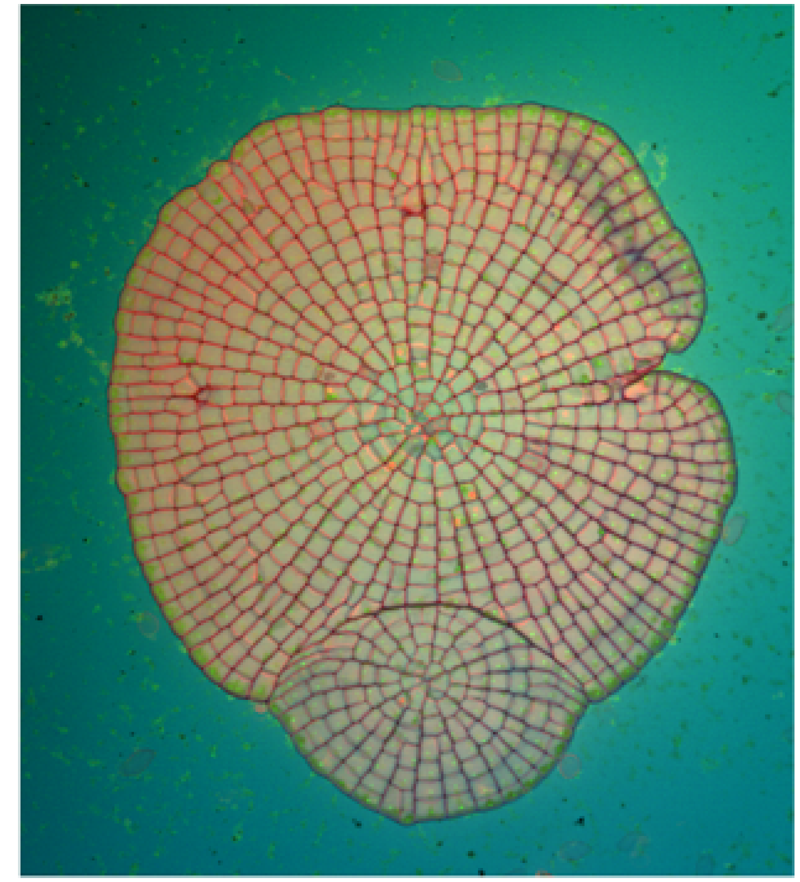
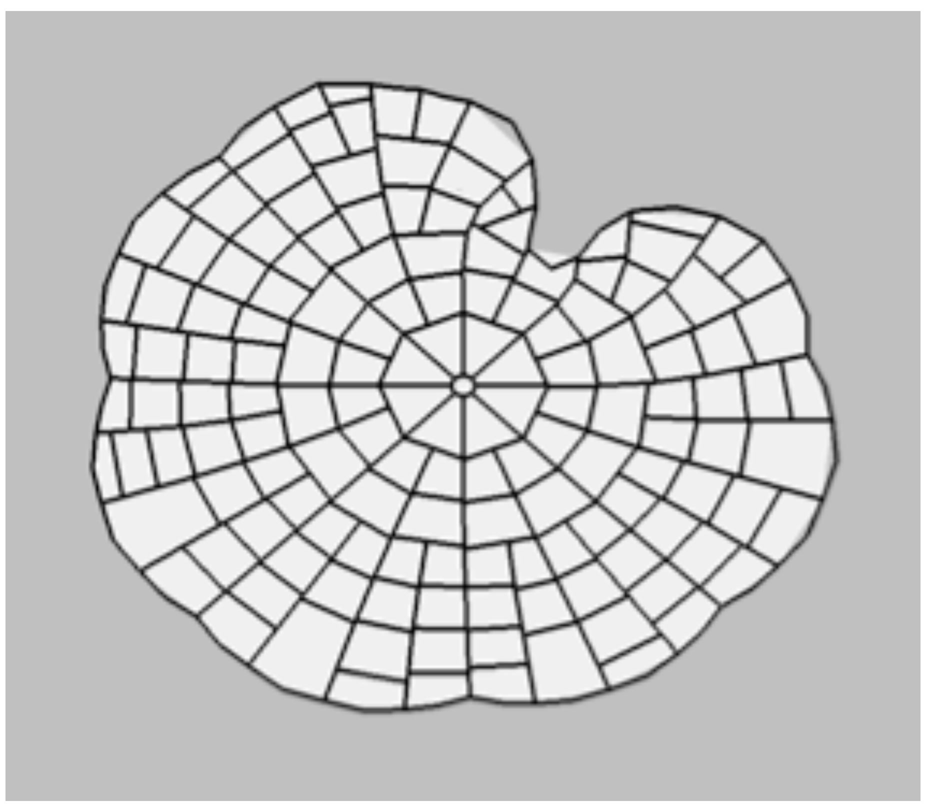
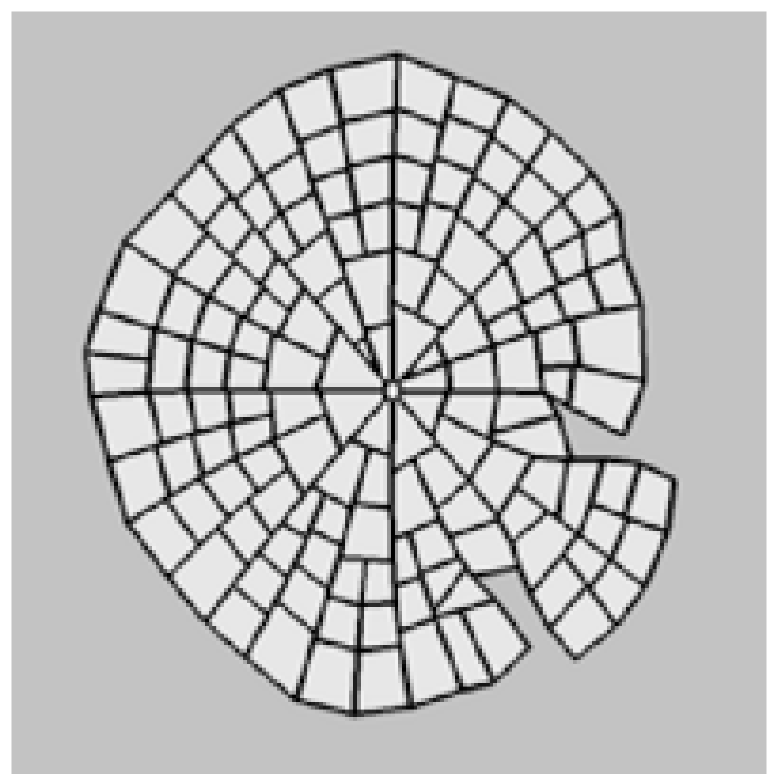
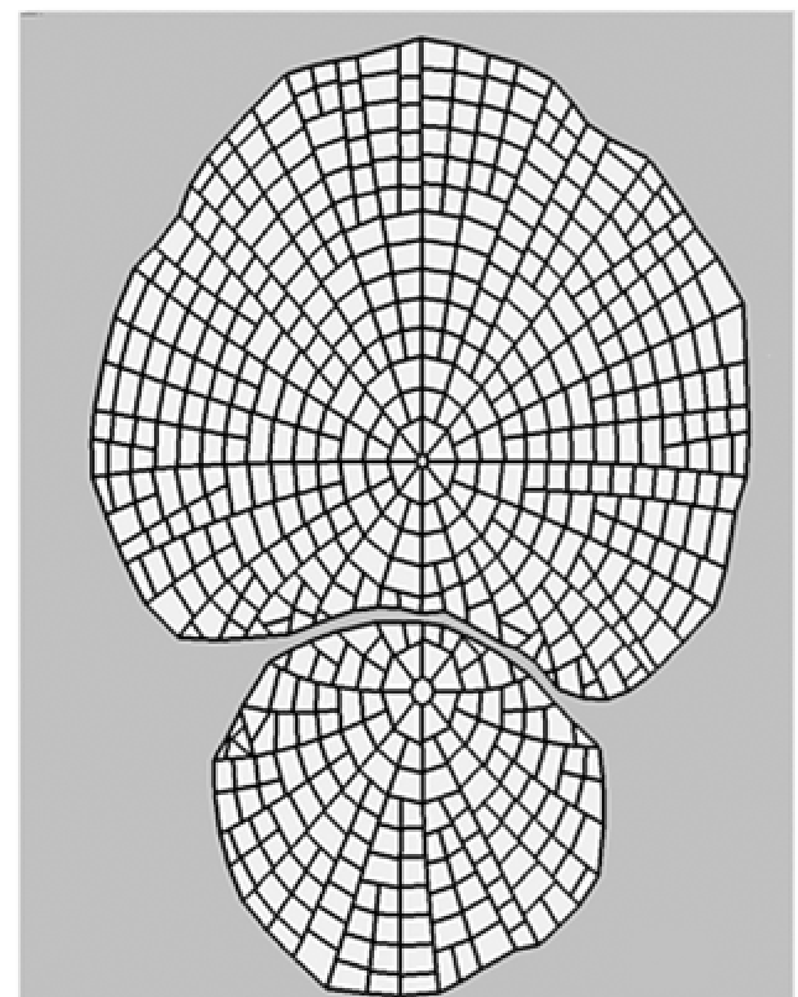
CellModeller for plant morphogenesis



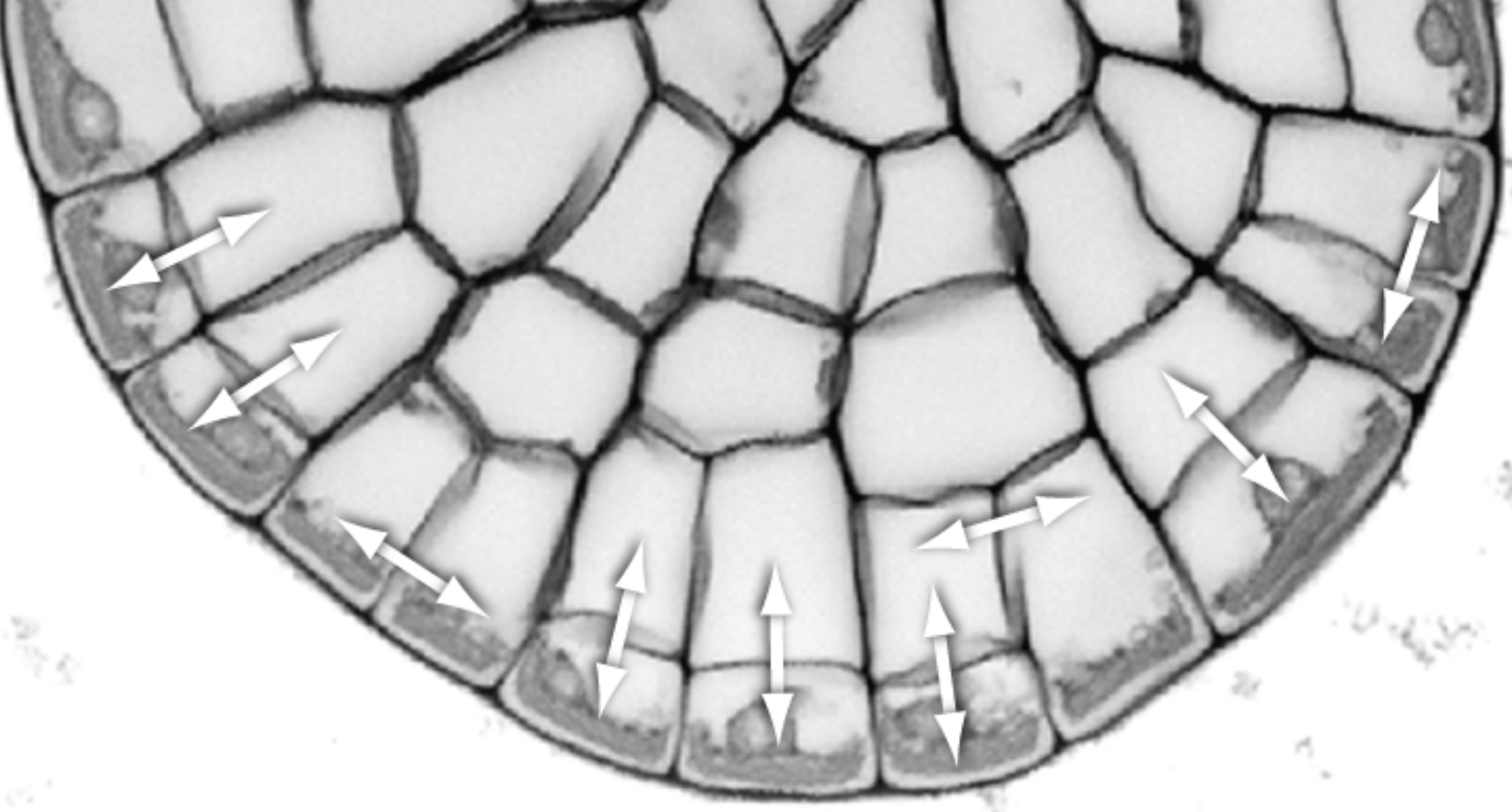


Tim Rudge,
Jonathan MacKenzie
& Lionel Dupuy

Lionel Dupuy

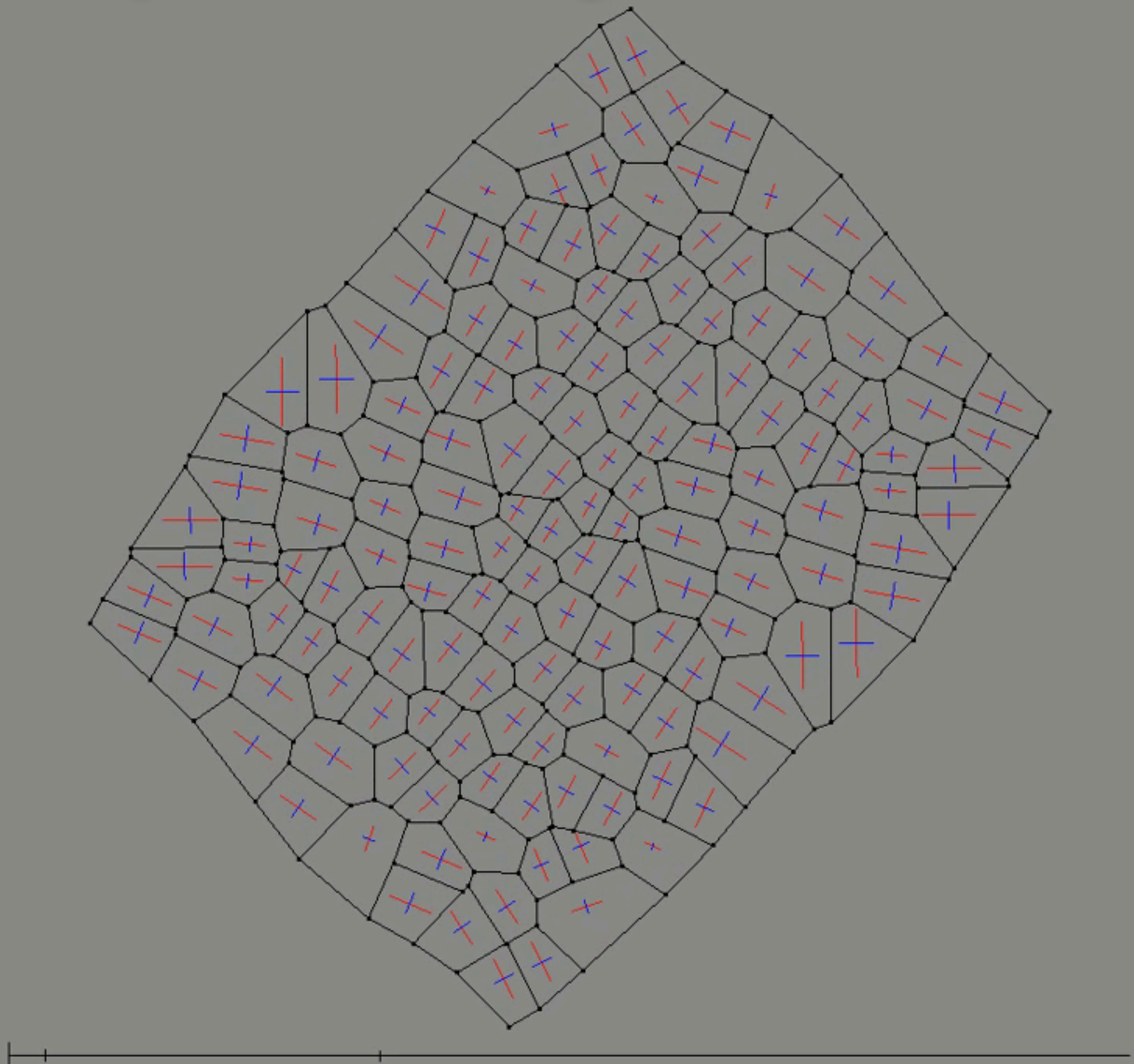







Modelling growth of Coleochaete

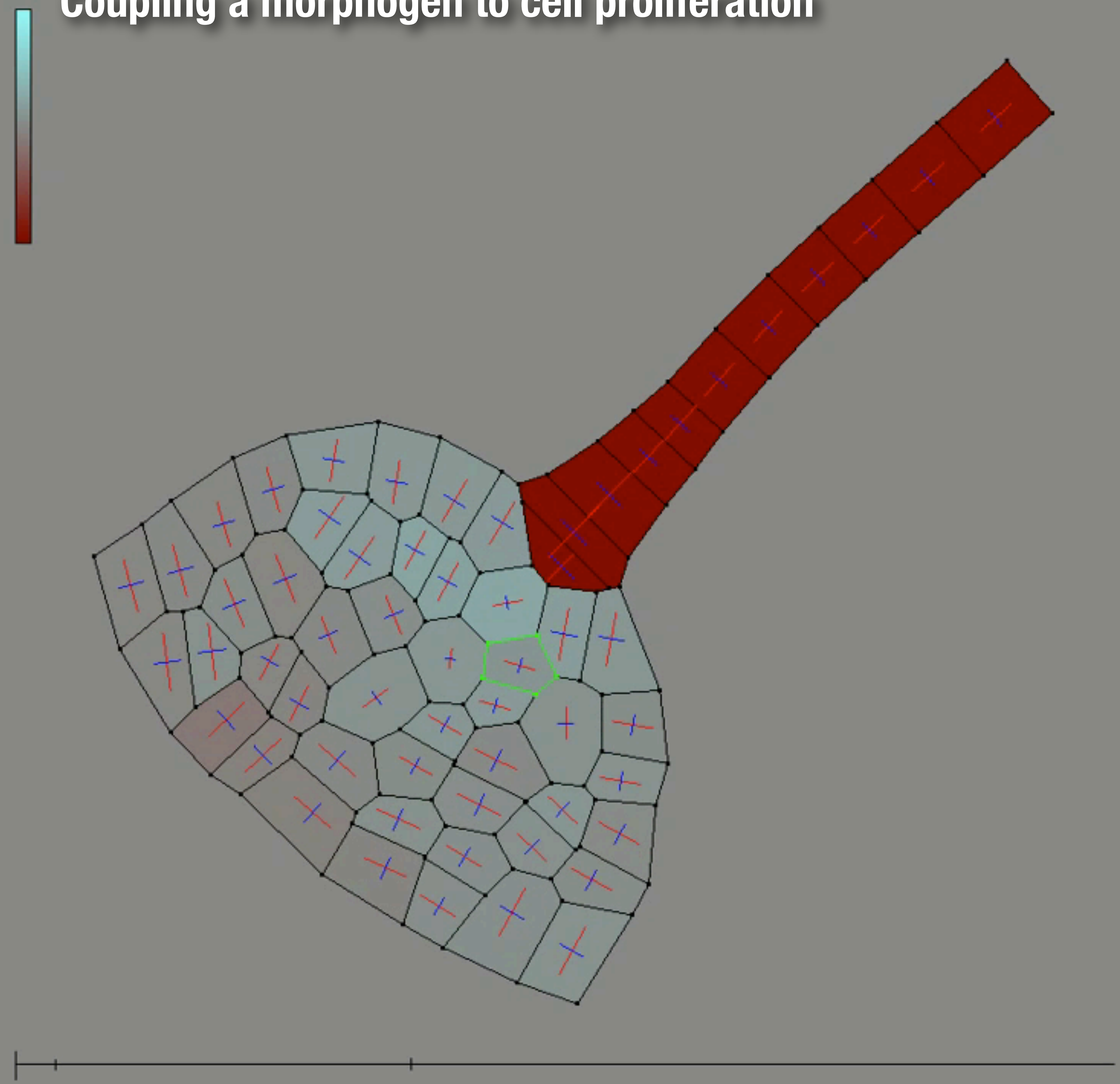


How are the planes of cell division determined?

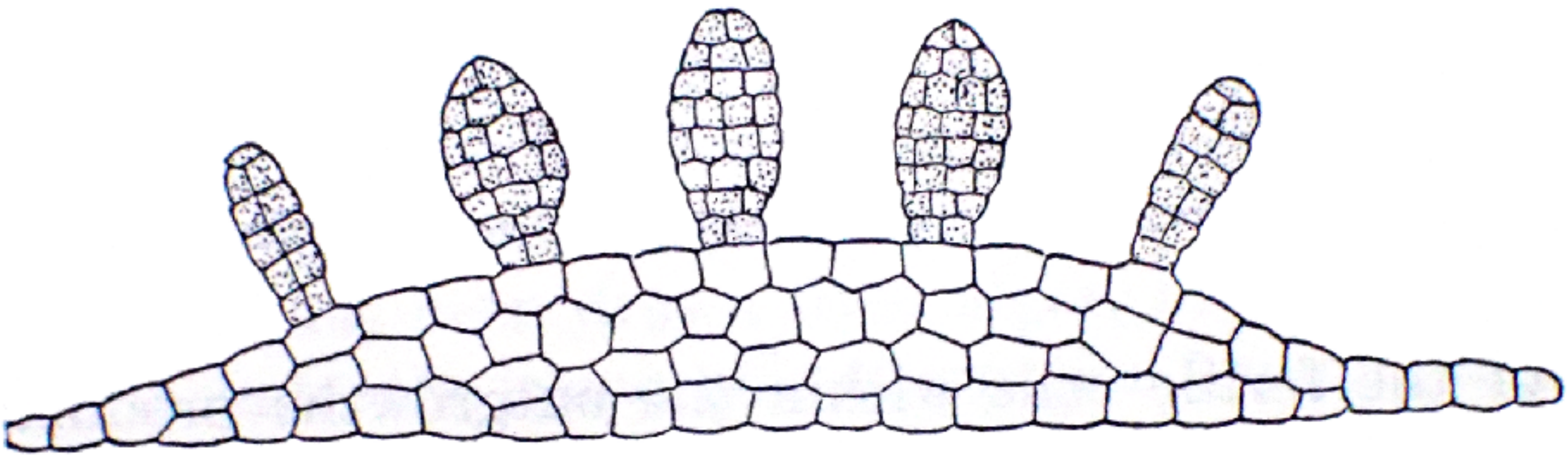
Computer model for cellular growth



Coupling a morphogen to cell proliferation



Primitive terrestrial plants

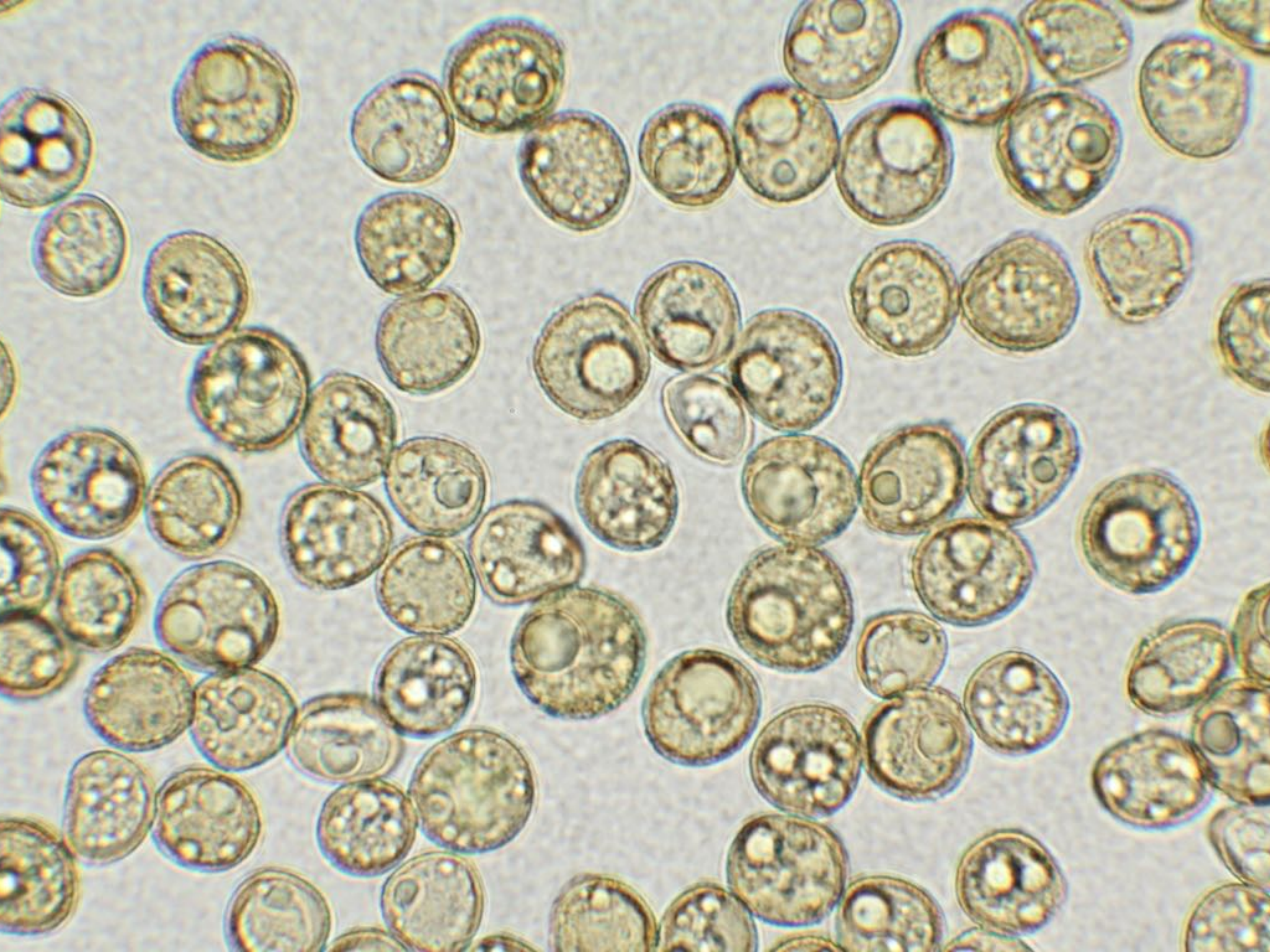


Speculation on the form/features of first terrestrial plants (~500Mya)

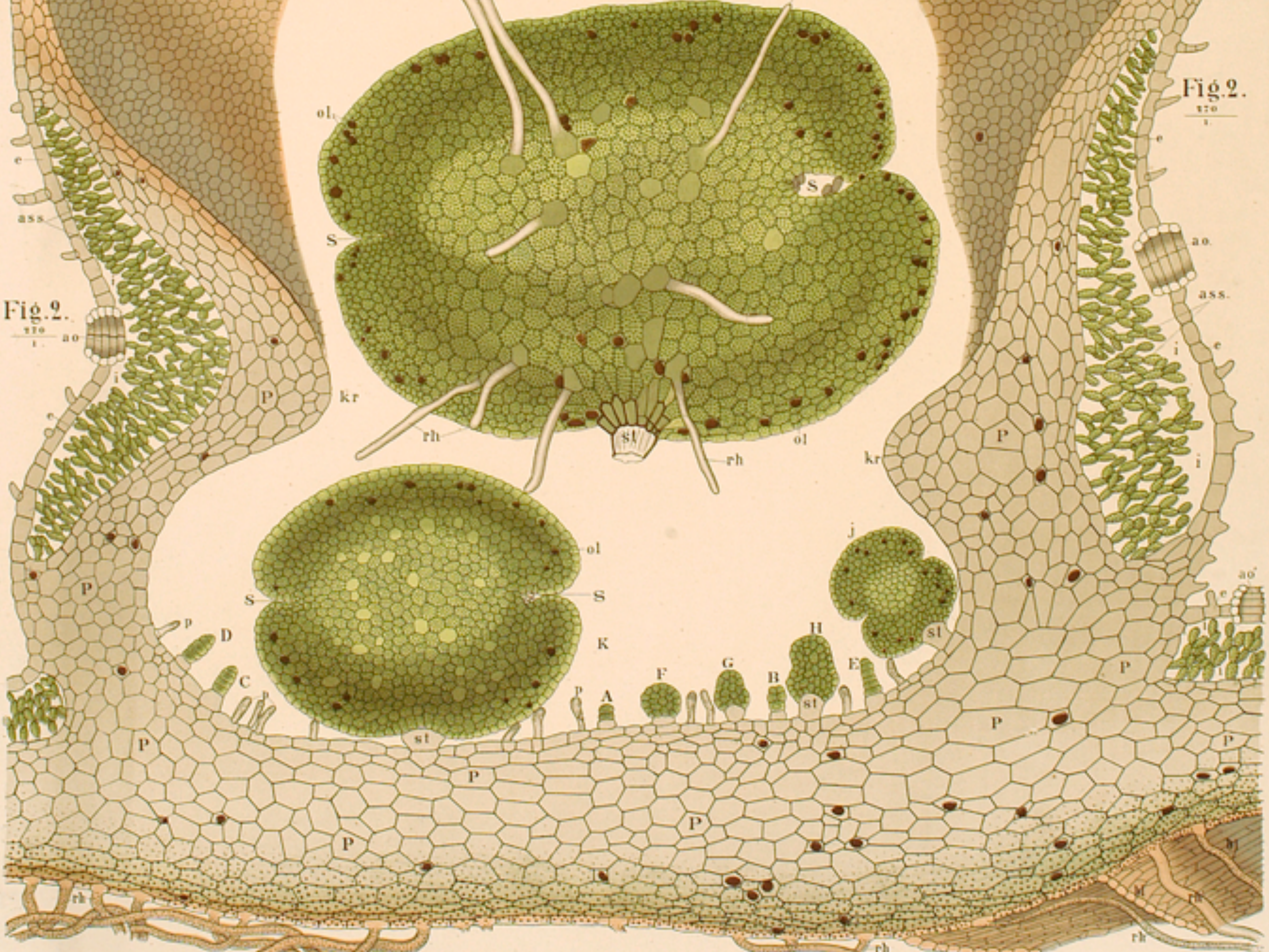
Cryptogamic Botany, Gilbert Smith 1955

A simple system for synthetic biology

Mature sporangia after crossing



Spontaneous production of clonal propagules

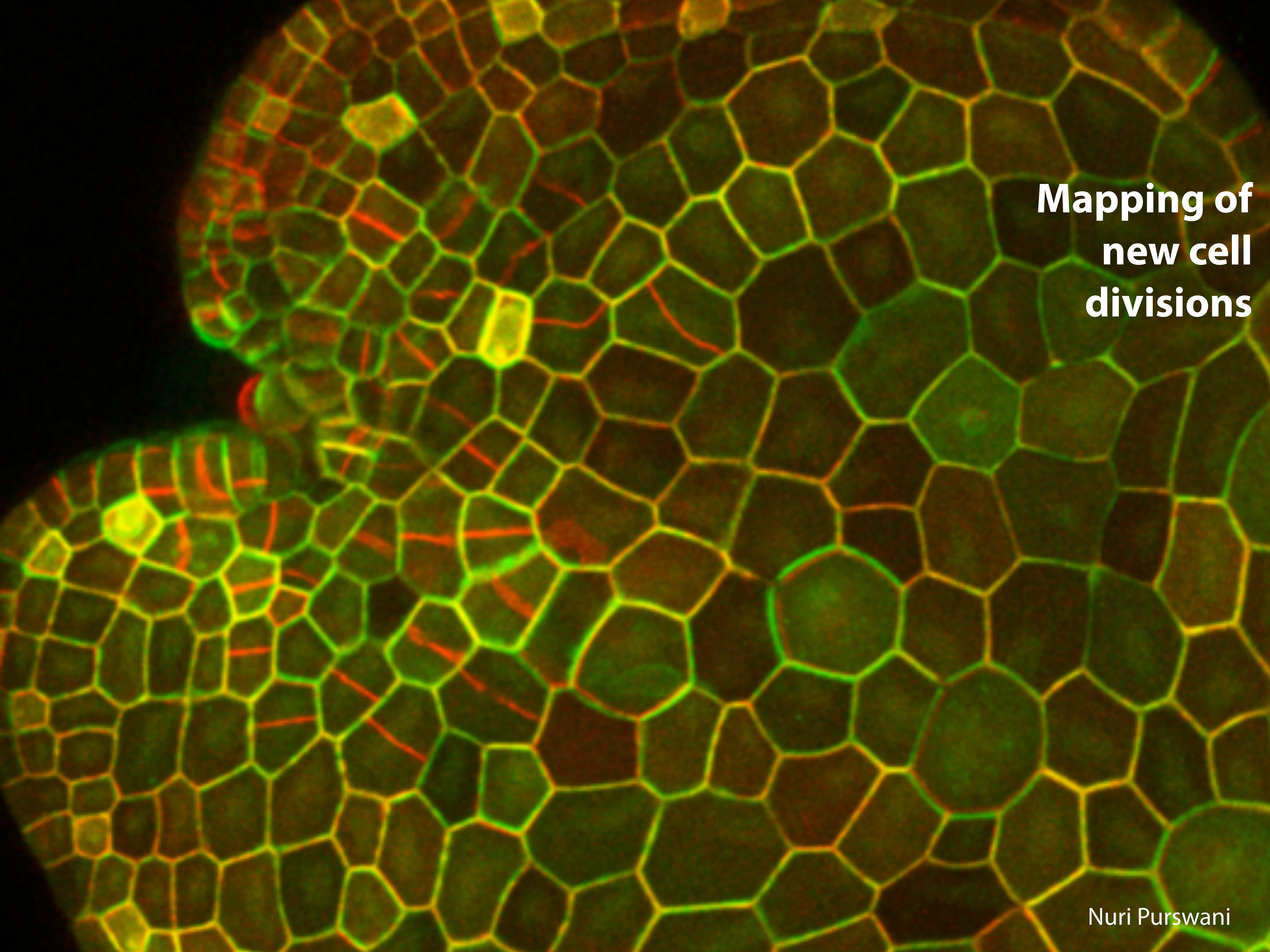


Marchantia polymorpha, L.

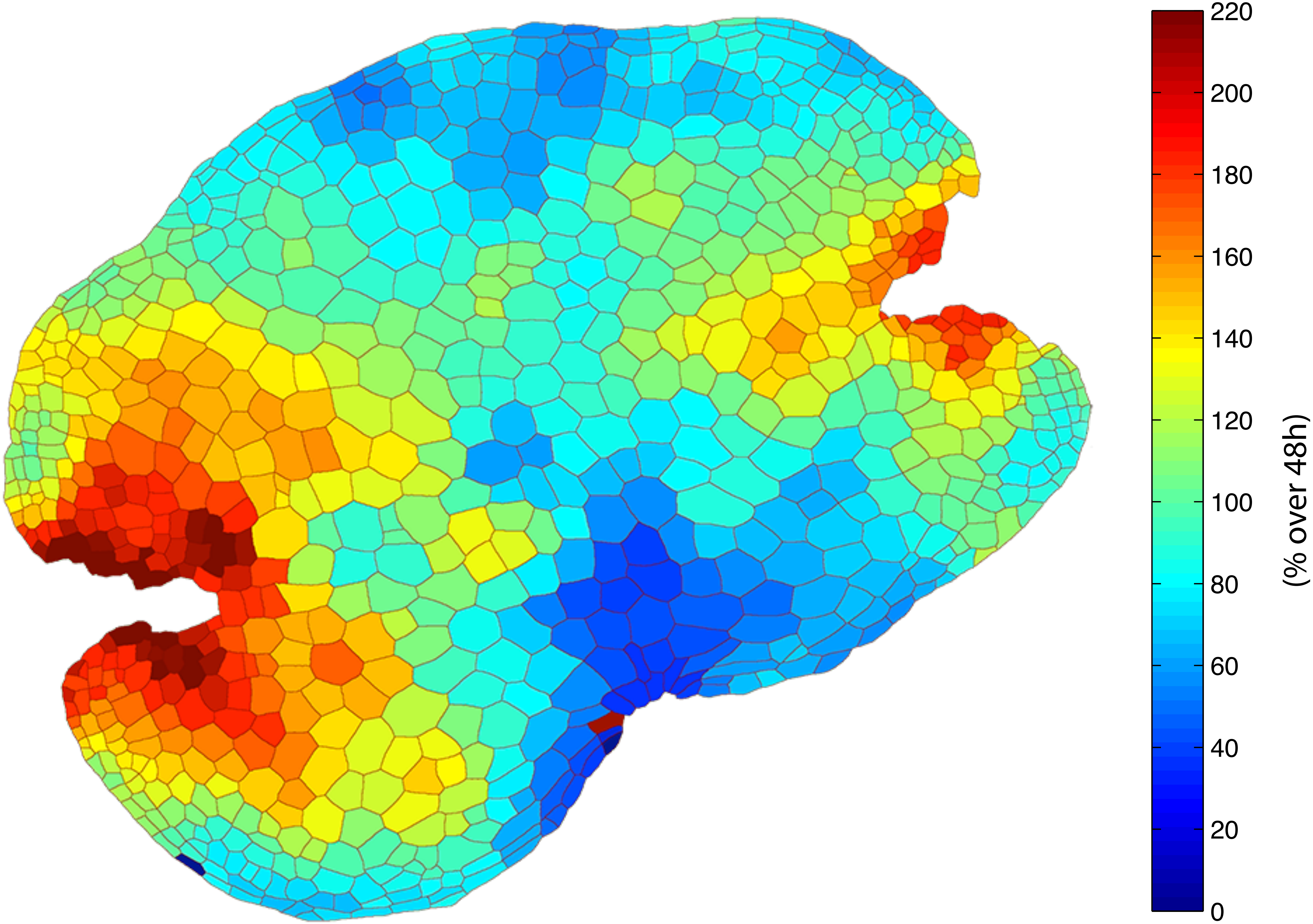
Arnold Dodel-Port ad nat. del.

J. F. SCHREIBER, ESSLINGEN. Edit.


100µm

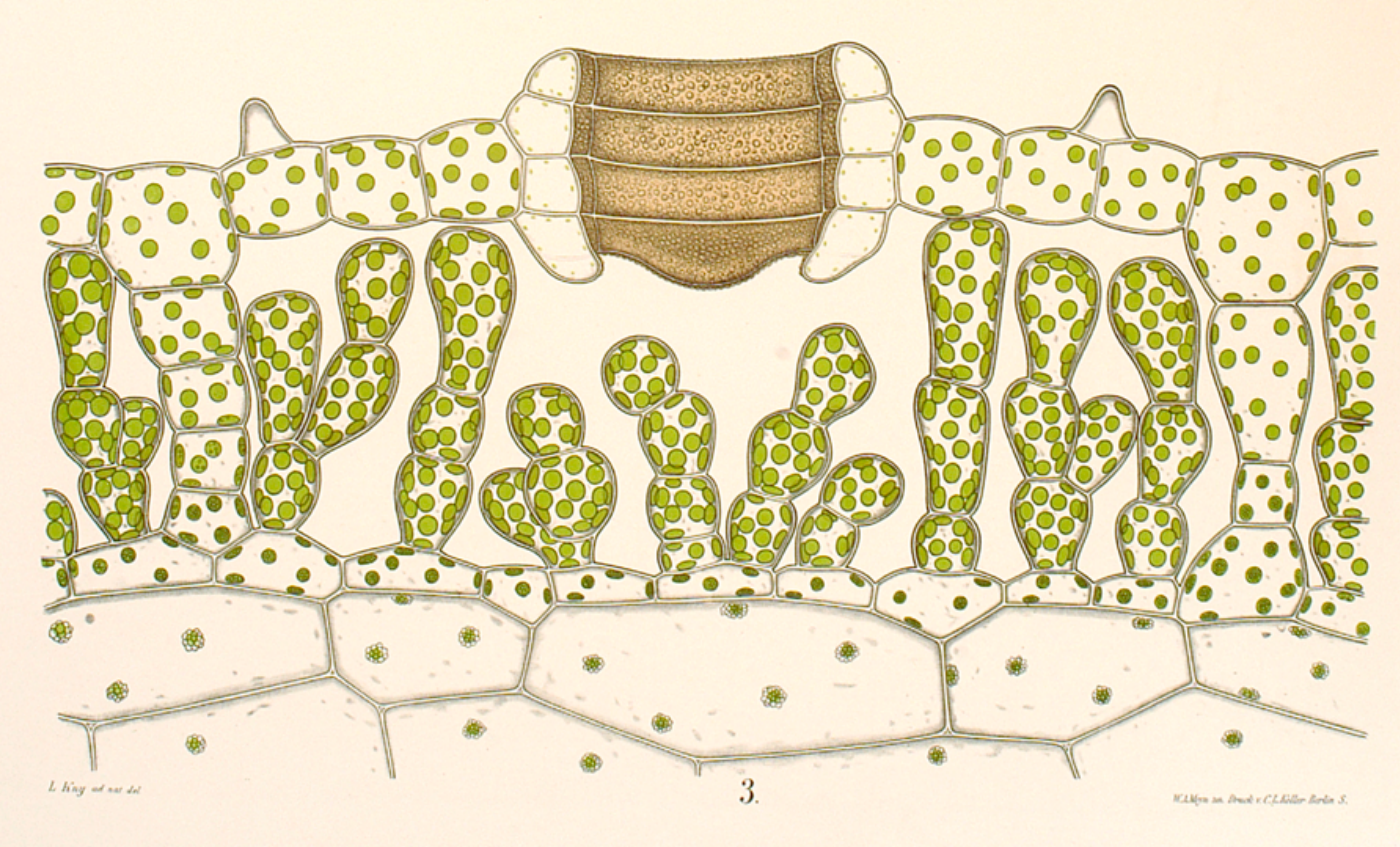

Mapping of new cell divisions
Nuri Purswani

Map of rates of cell expansion in *Marchantia polymorpha* gemma

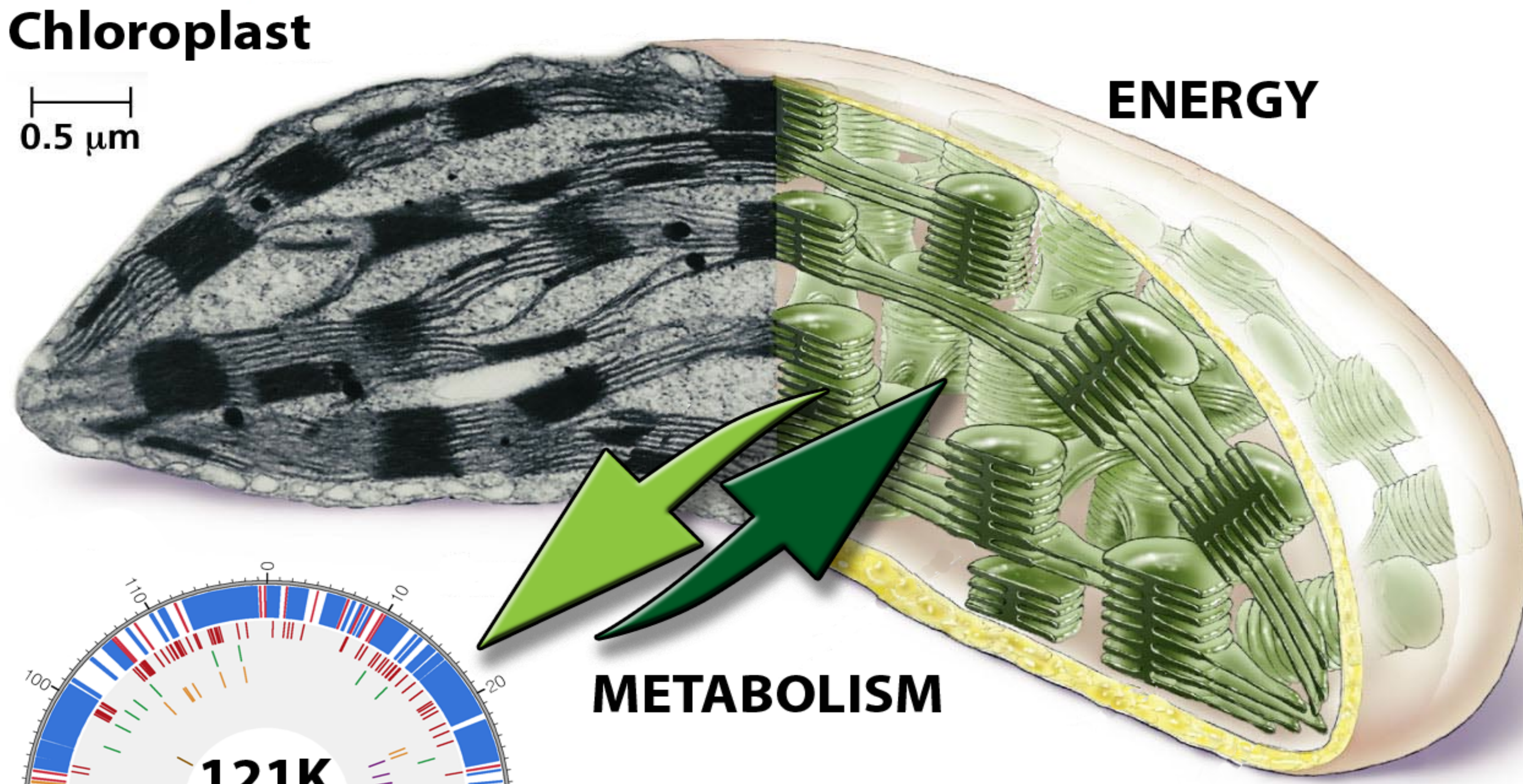


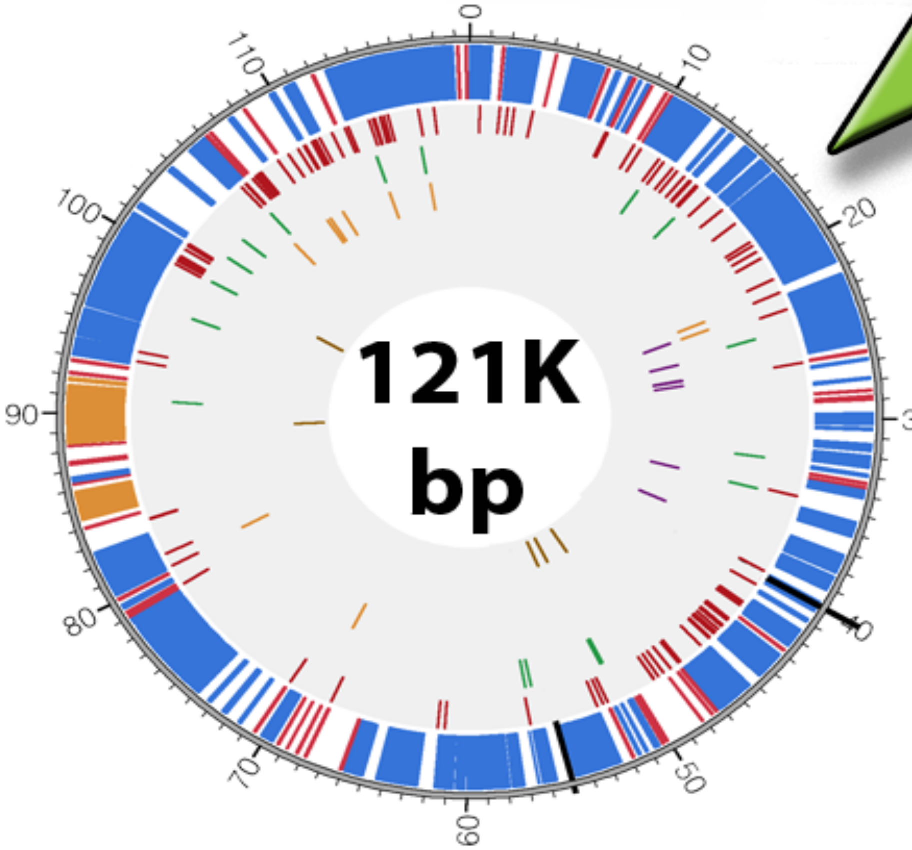
Nuri Purswani

Simple and modular patterns of growth



Transverse section of Marchantia polymorpha thallus (Leopold Kny)





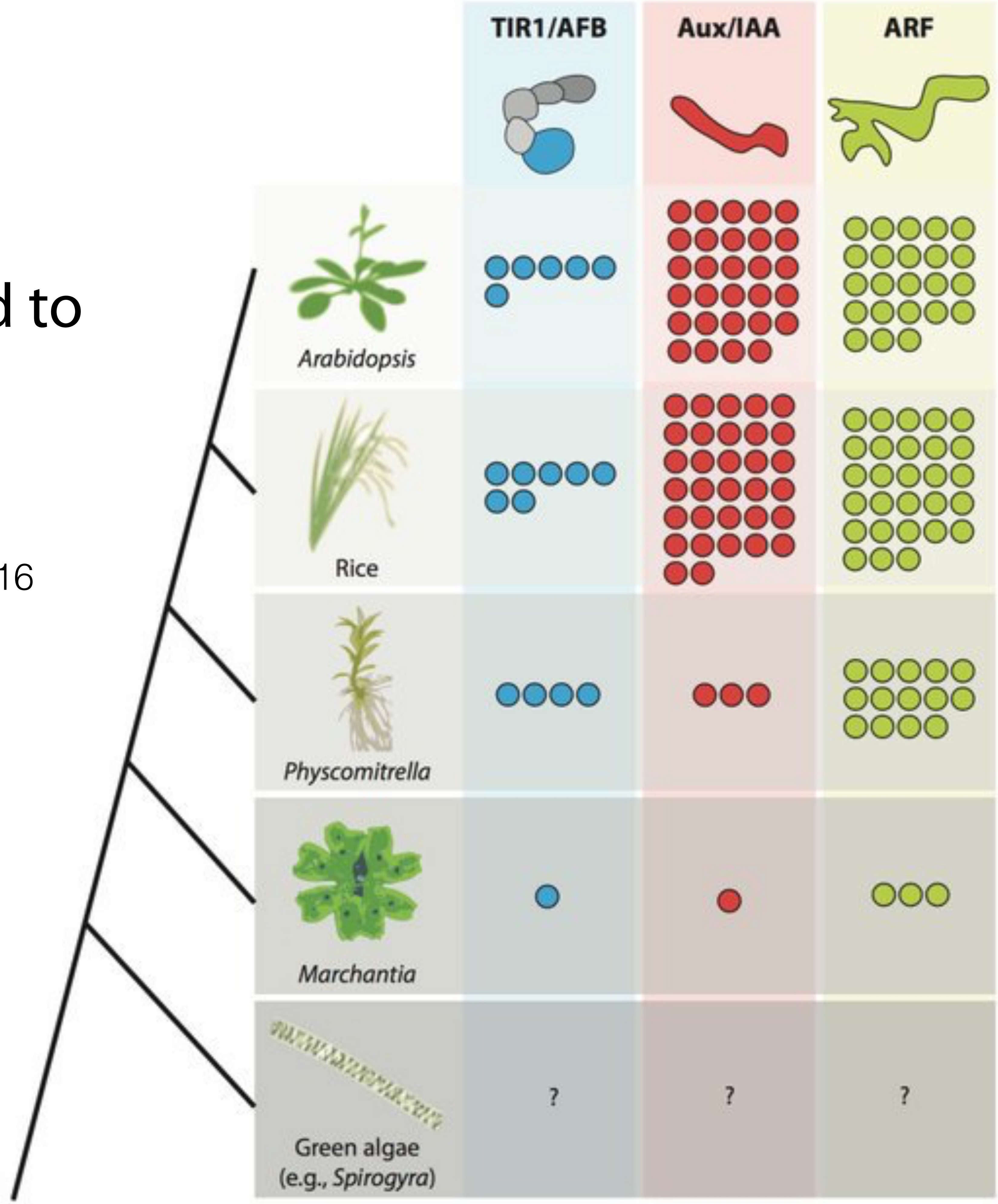
Synthetic plastid genome

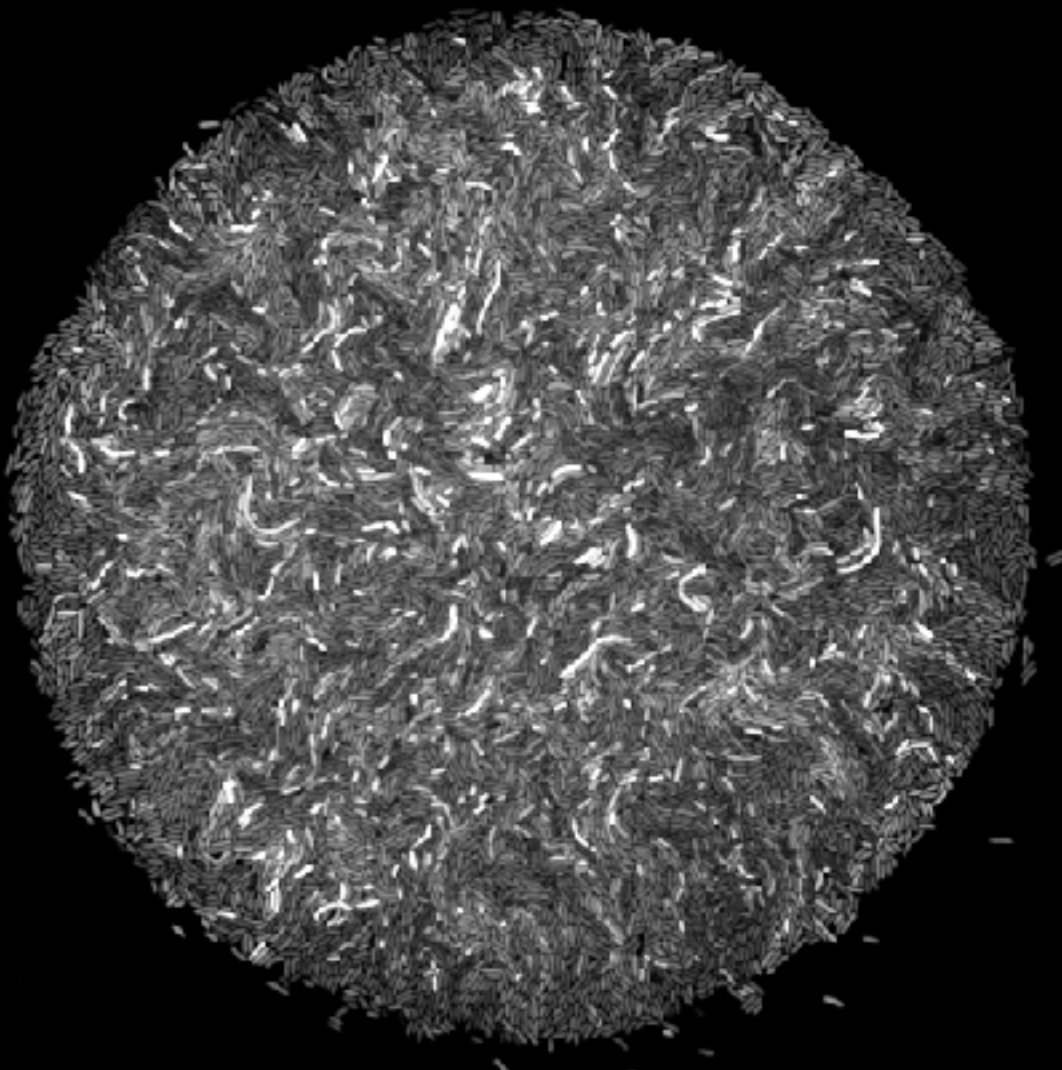
Small with prokaryote signals
Direct access to source of plant energy and metabolites
Yeast-Plant shuttle for reprogramming plant metabolism

Reduced gene sets

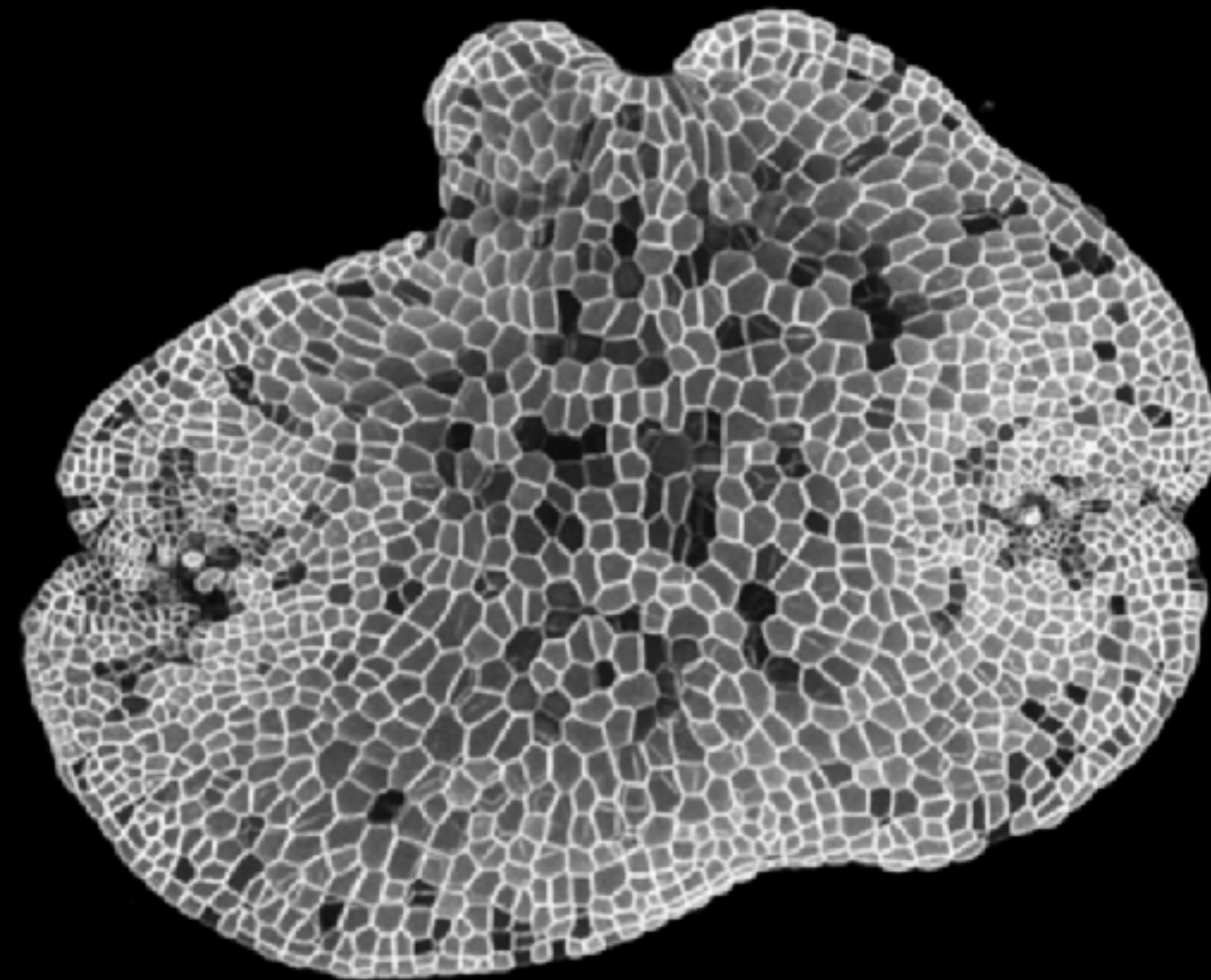
e.g. number of auxin response genes in Marchantia compared to higher plants

Dolf Weijers and Doris Wagner
Transcriptional responses to auxin
Ann Rev Plant bill. 67:539-574, 2016



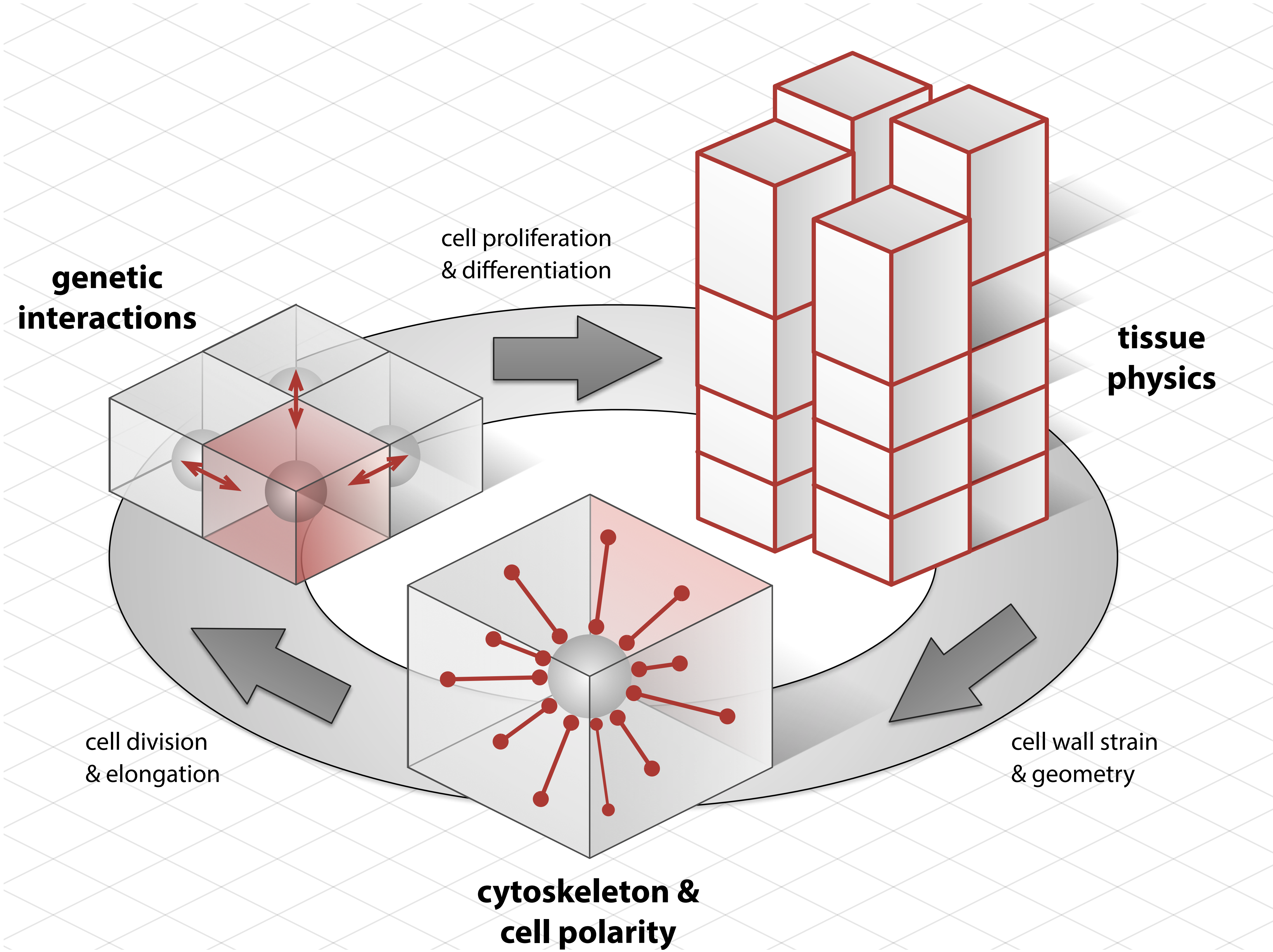


Bacteria



Marchantia

Simple models for multiscale engineering of cell populations



Multi-scale view of plant growth. (i) Interaction between cytoskeletal elements and local cell wall determinants, strain or geometry regulates the polarity of cell division and elongation. (ii) Genetic interactions between neighbouring cells trigger gene expression, cell proliferation and differentiation. (iii) Cellular growth results in physical strains that are transmitted across tissues and constrain cell growth. (iv) Physical constraints on cell size and shape regulate timing and orientation of individual cell divisions and guide morphogenesis.

Reprogramming plants

- Plants provide proven, global, low-cost technology for gigatonne scale bioproduction
- We need faster, simpler multicellular systems for engineering form and metabolism
- Synthetic biology offers breakout technologies

Lecture 1: Genetic modification in agriculture and the advent of Synthetic Biology.

Lecture 2: Genetic circuits and genome scale DNA engineering.

Lecture 3: Engineered logic and the control of gene expression.

Lecture 4: Self-organisation and reprogramming of multicellular systems.

1. **Organisation of natural microbial populations**
2. **Natural cell-cell signalling systems**
3. **Cellular coupling of genetic circuits**
4. **Physical coupling of cell populations**
5. **Organisation of plant cell divisions**
6. **Simple plant systems**
7. **Potential for plastid engineering in plants**
8. **Multiscale modelling**
9. **Prospects for plant engineering**

Additional resources: **http://www.haseloff-lab.org** (Education)