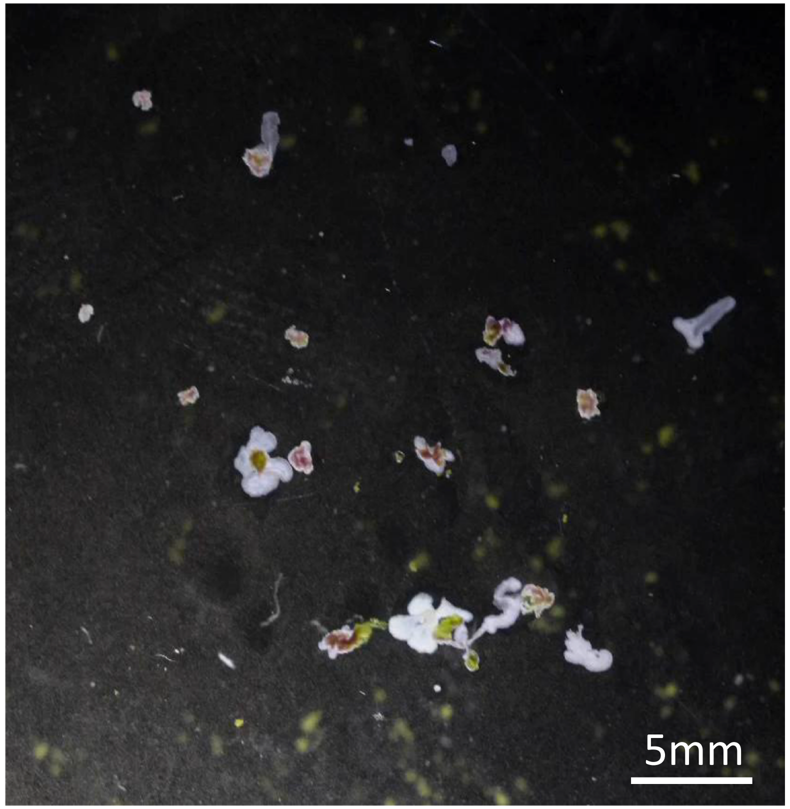
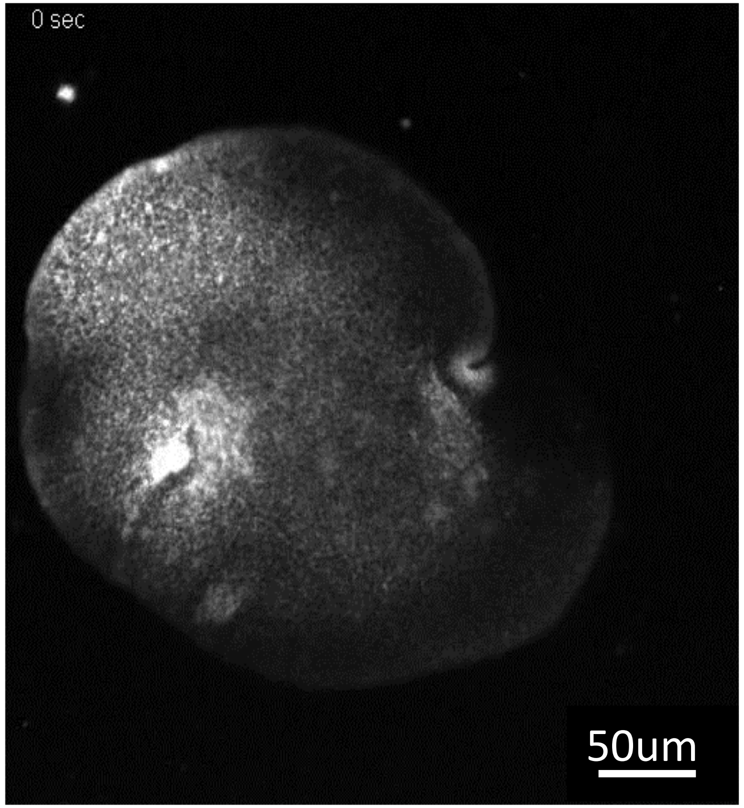
How can tissues actively avoid rupture?
(lessons from Trichoplax)



15x fast



10x fast

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KITP 2019

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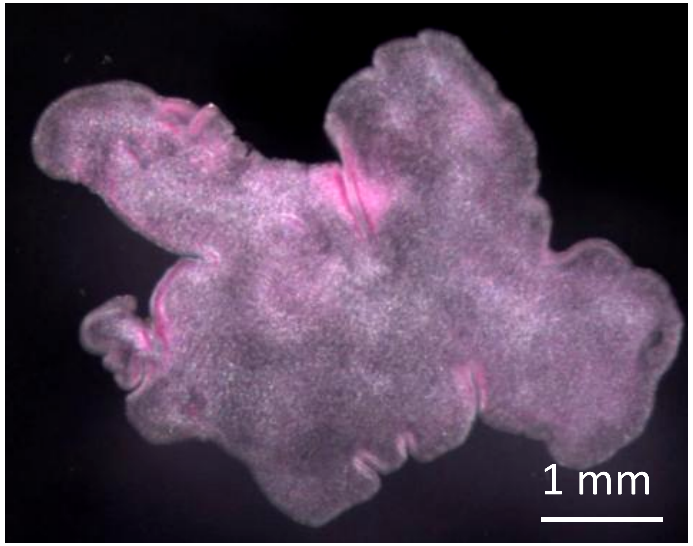
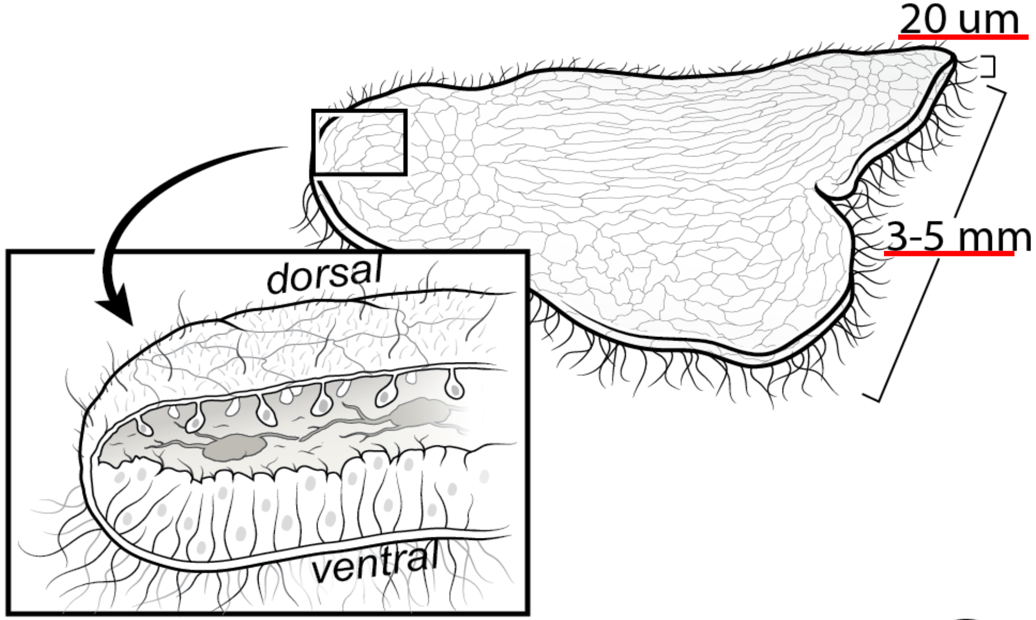
Stanford University

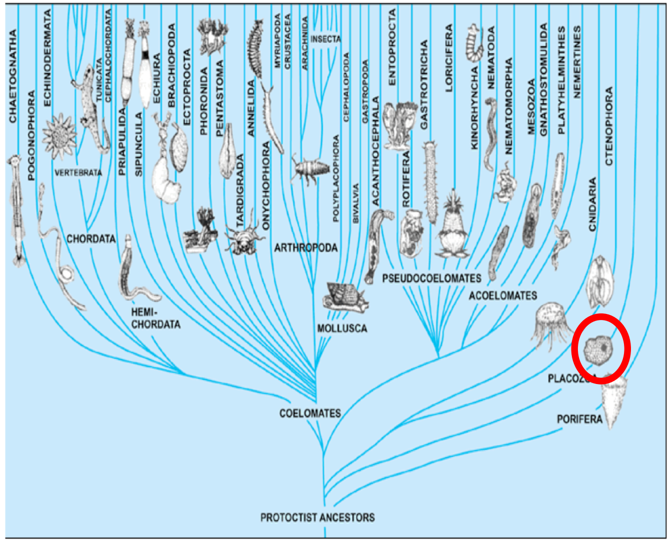
Prakash Lab

Trichoplax Adhaerens/Placozoa (Tplax)





Animal kingdom evolution



claimed simplest living animal

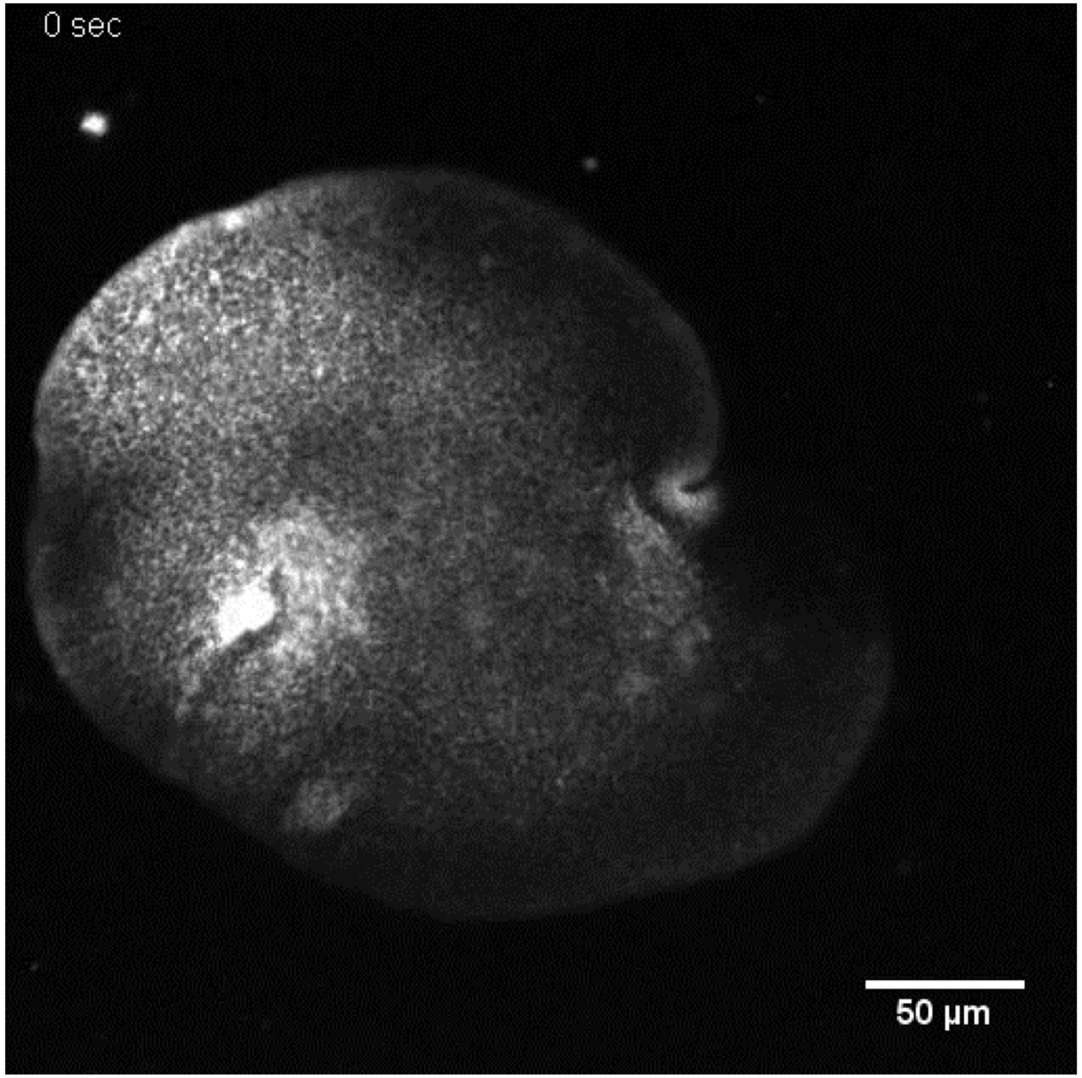
- 2D pancake, minimal symmetry breaking
- No neurons or muscles
- Two epithelial layers
- No Extra-cellular-matrix
- Only adherens junctions

but exhibits complex behaviors:

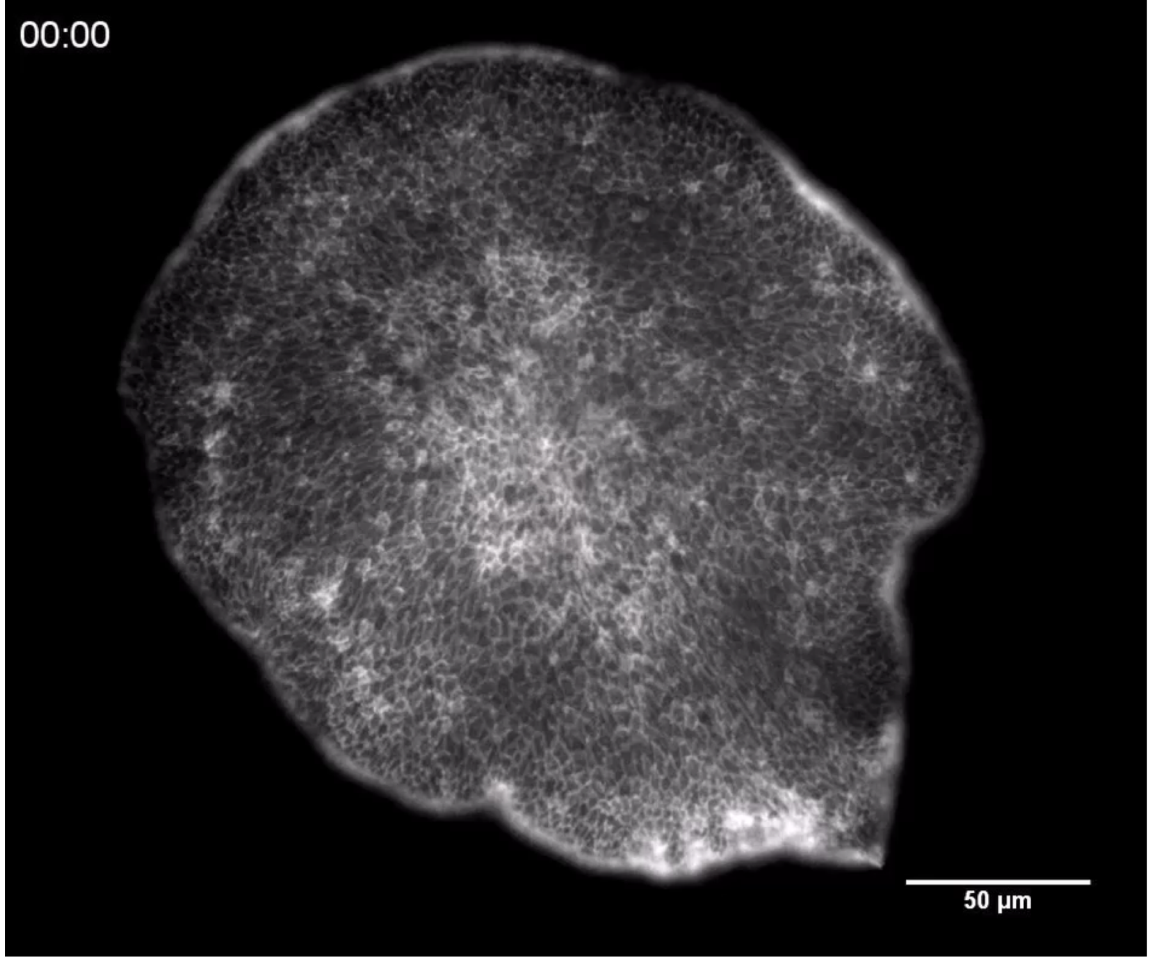
-Directed locomotion
-Taxis
-External digestion
-Division by fission

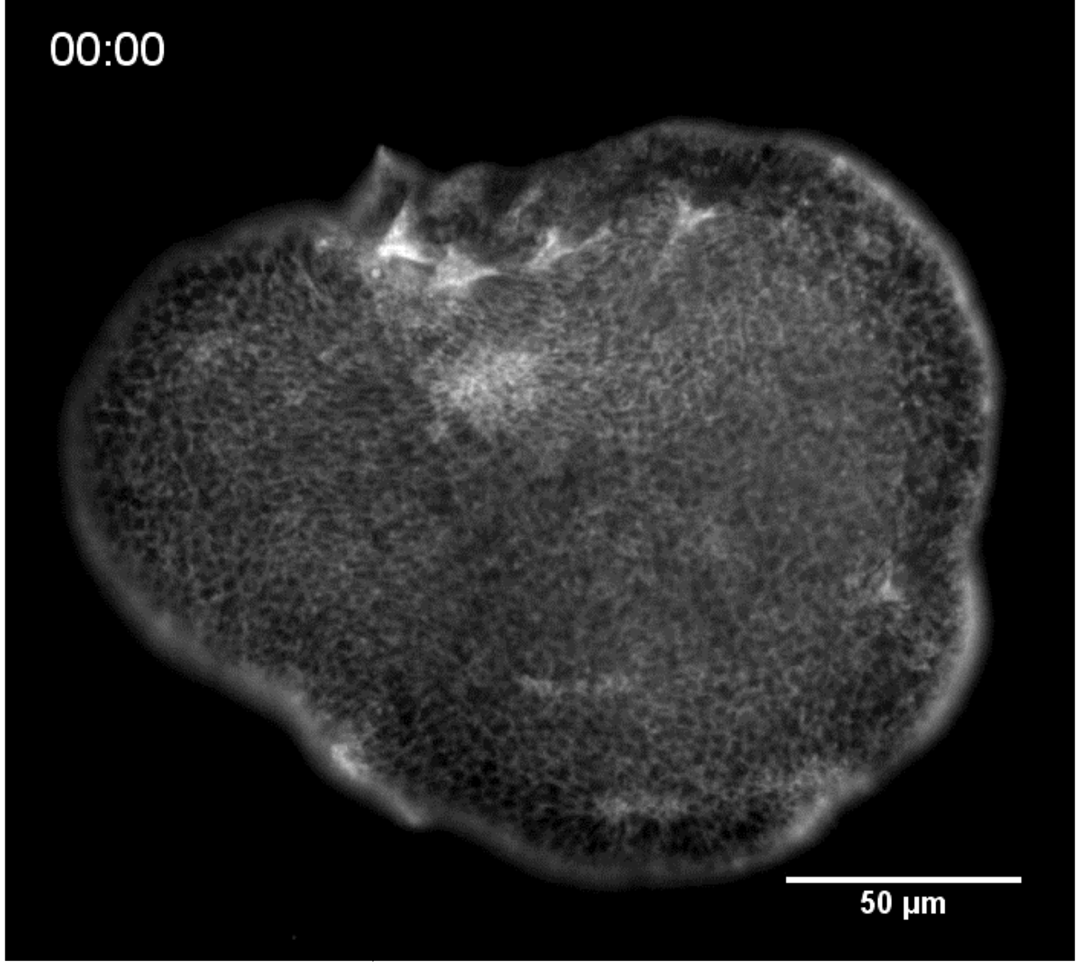
How does the animal coordinates itself??

Live Tracking from top view:

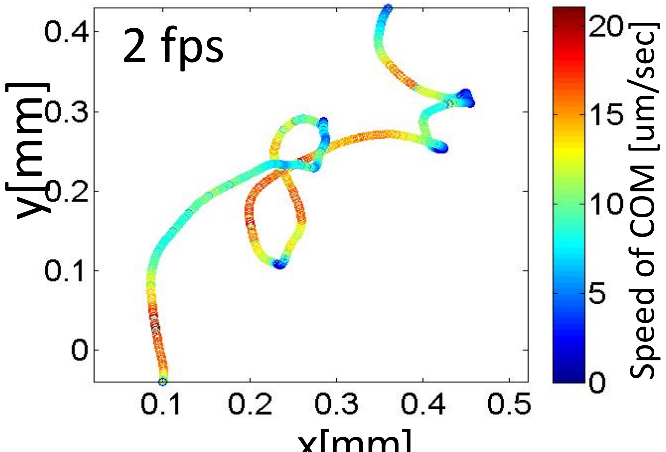


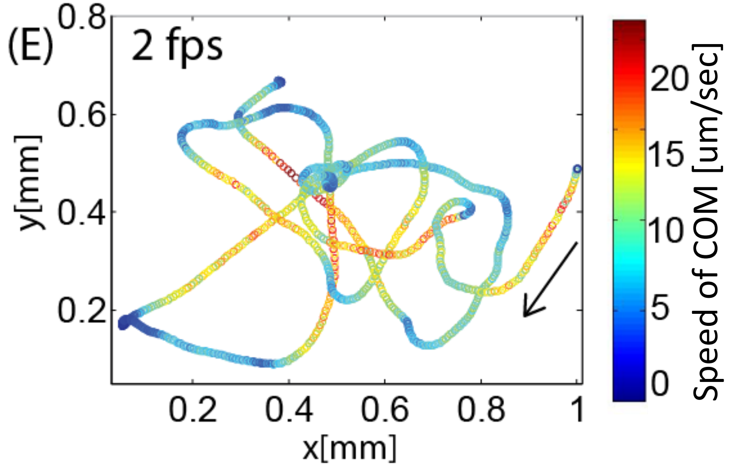
CMO live membrane stain
10x fast

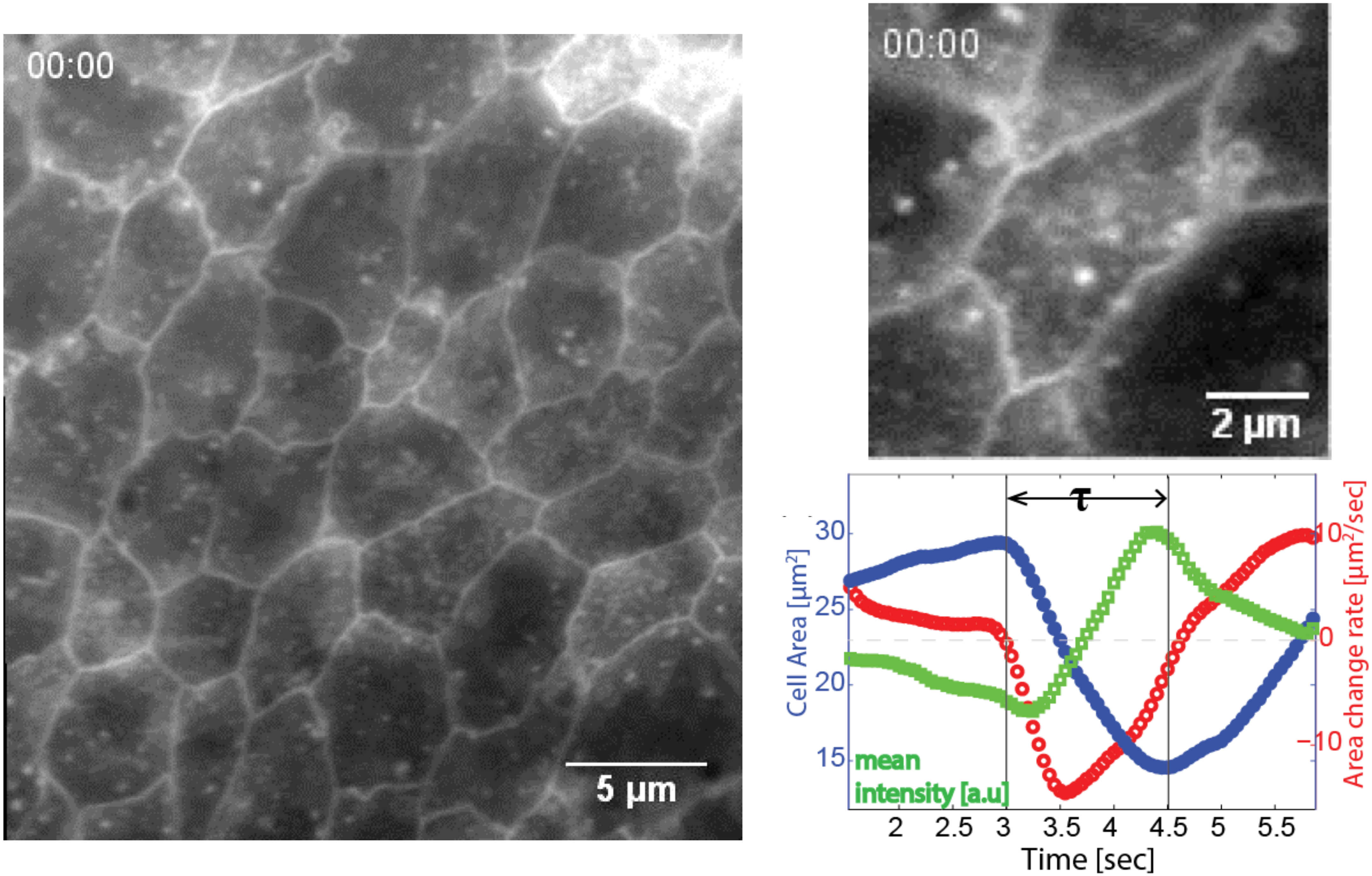




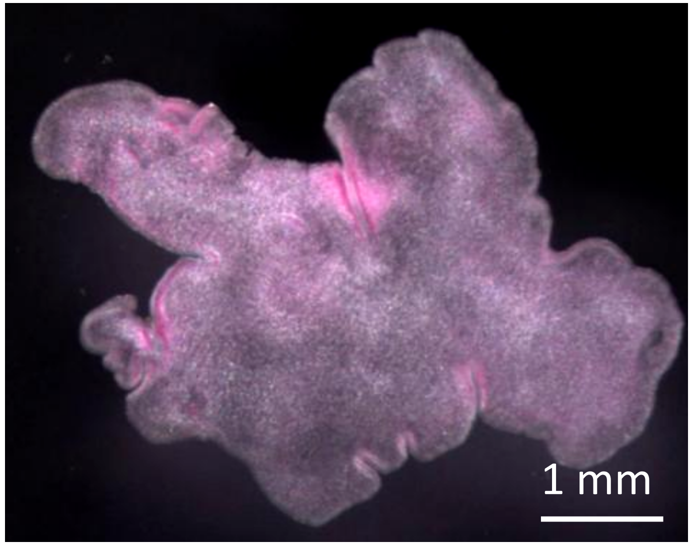
center of mass
Trajectory:







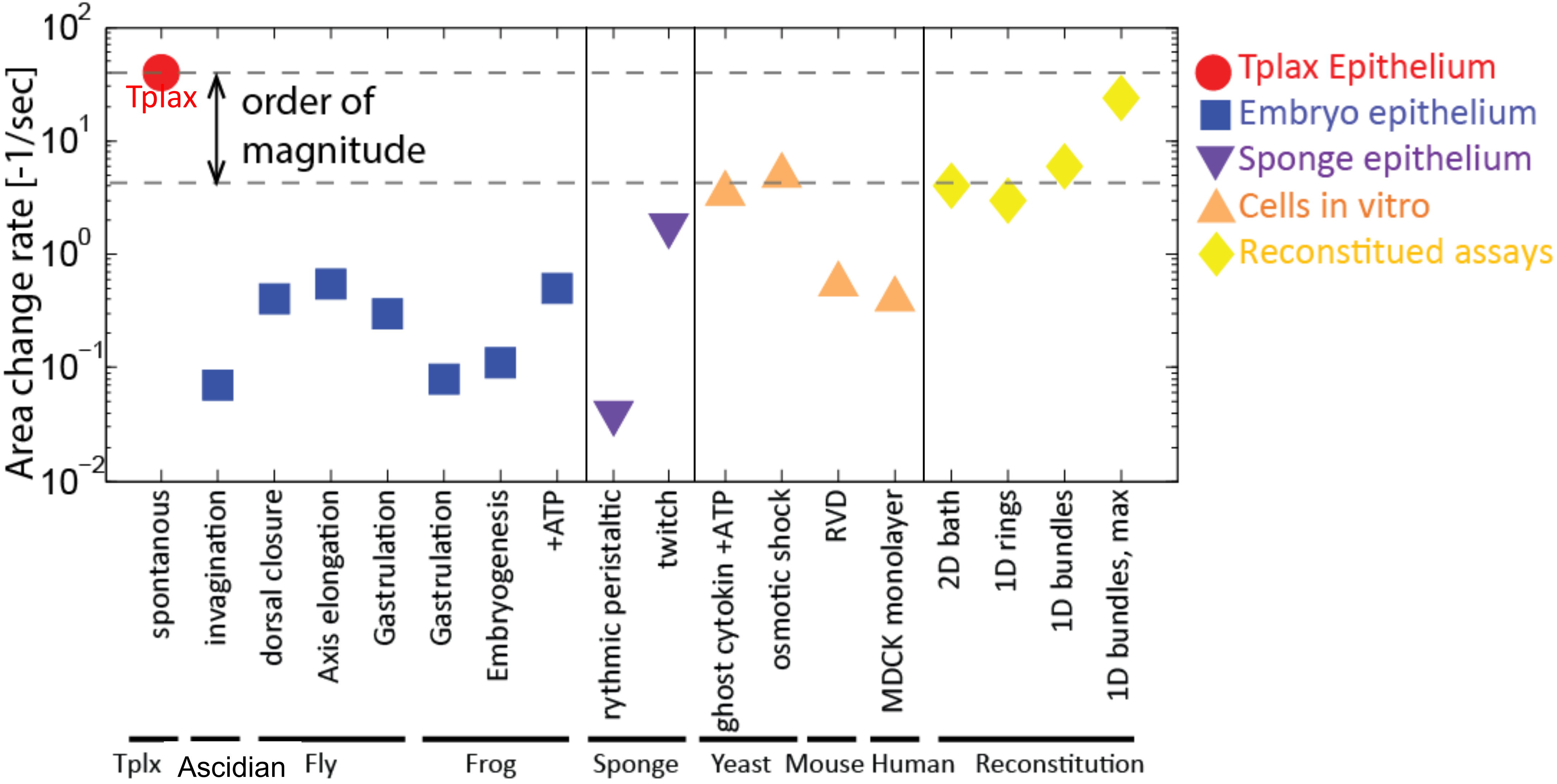
00:00
5 μm
00:00
2 μm
τ
Cell Area [μm²]
30
25
20
15
mean
intensity [a.u]
Area change rate [μm²/sec]
10
0
−10
2
2.5
3
3.5
4
4.5
5
5.5
Time [sec]



Contraction Dynamics in *Trichoplax Adhaerens*

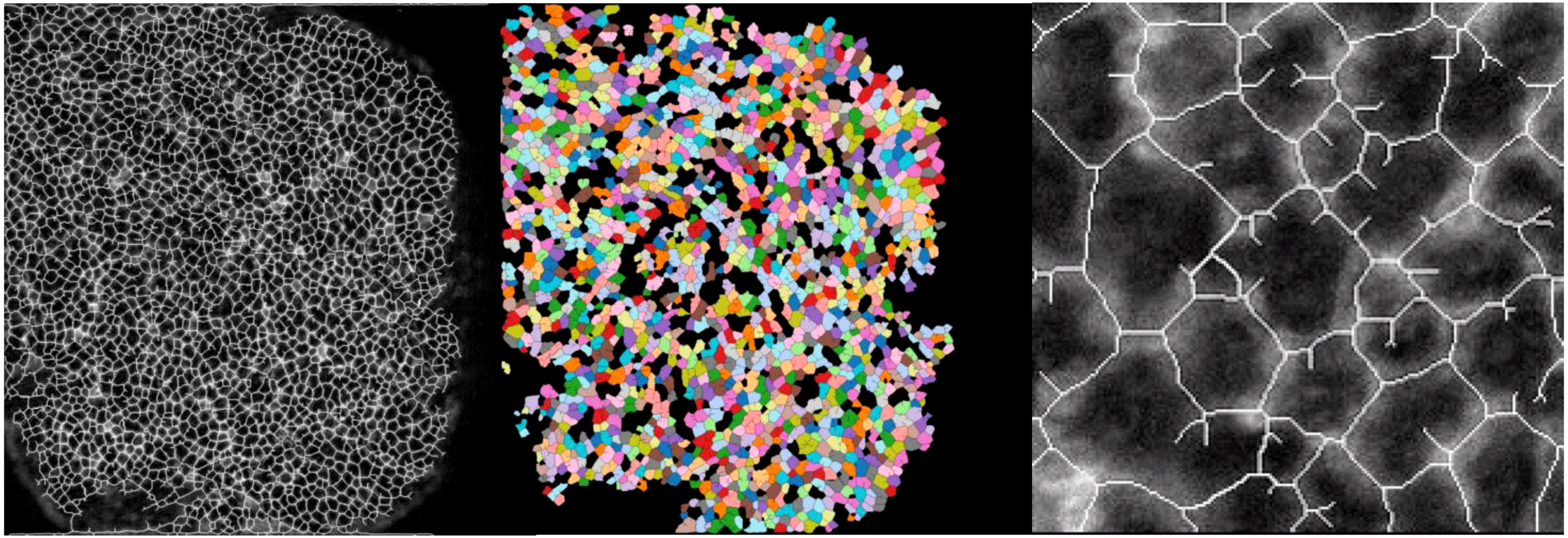
1. Intro: the "simplest living animal"
2. Story #1: High-speed of single cell contraction
3. Story #2: Tissue dynamics and the "active cohesion" hypothesis

Fastest Epithelial Contractions in the Animal Kingdom

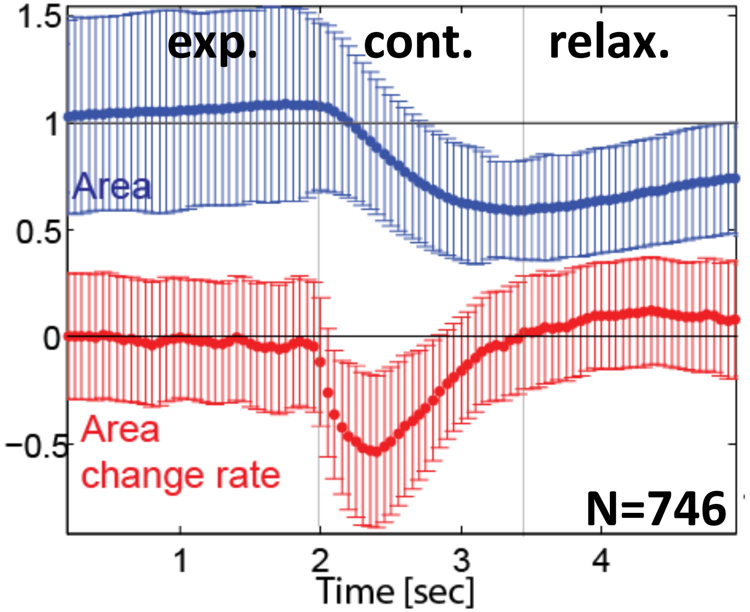


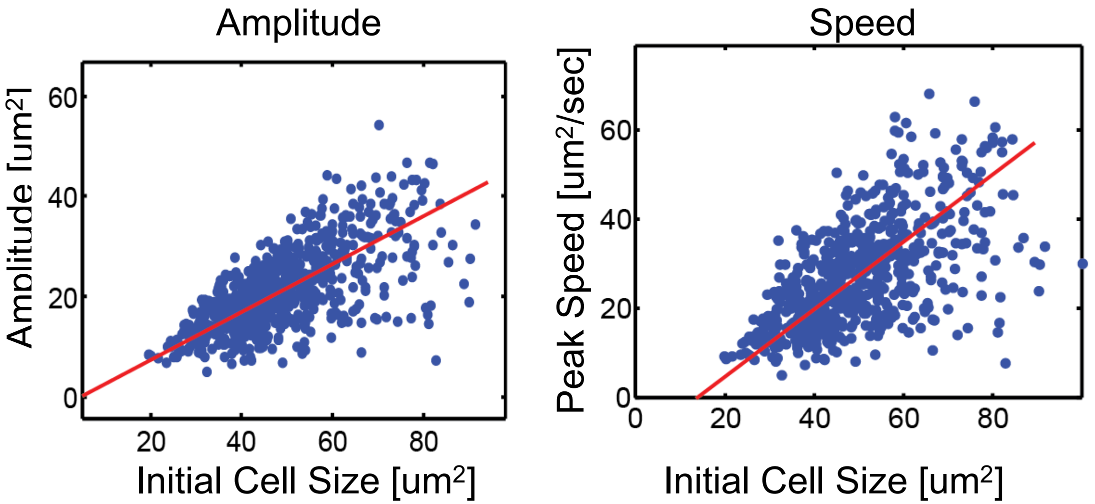
How come Tplax cells are so fast? or: Why all other cells so slow?

Collecting Statistical Data:



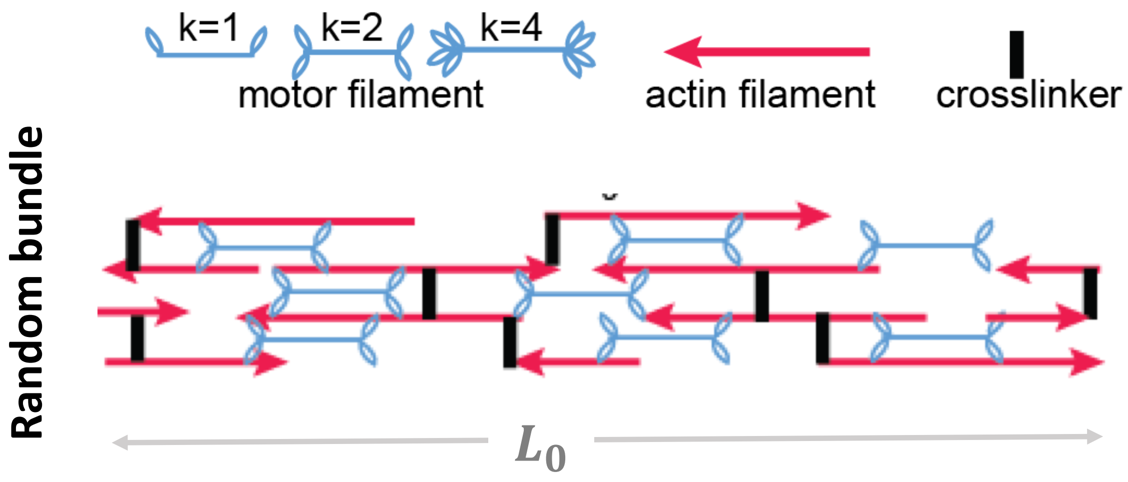
N=746 contraction events

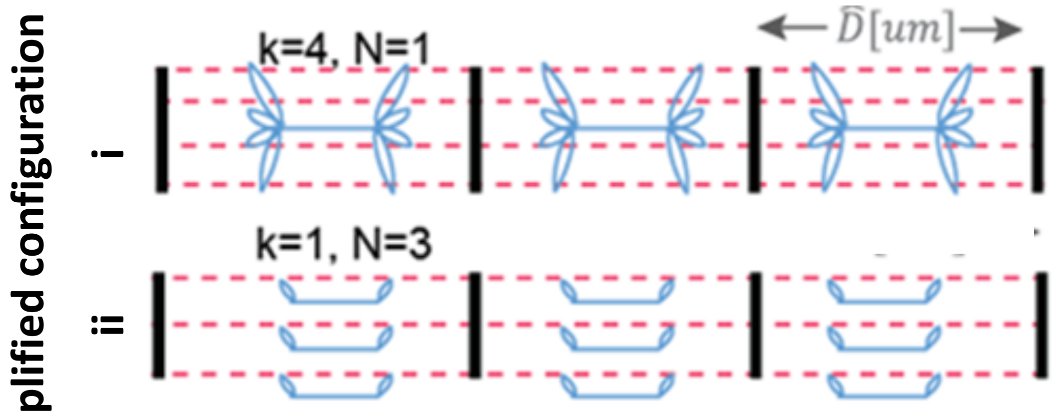




Armon et al, PNAS 2018

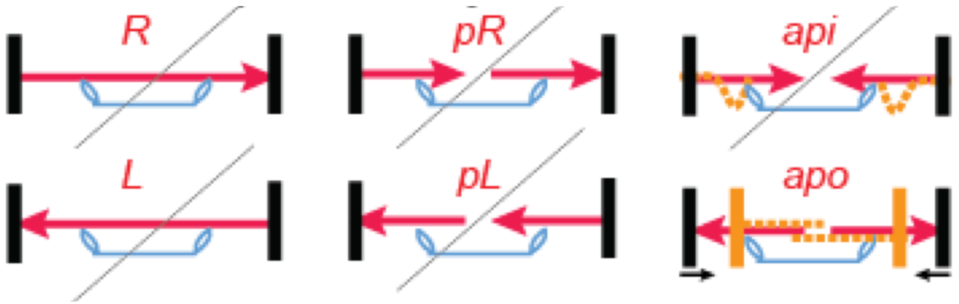
Random orientation 1D contractility speed



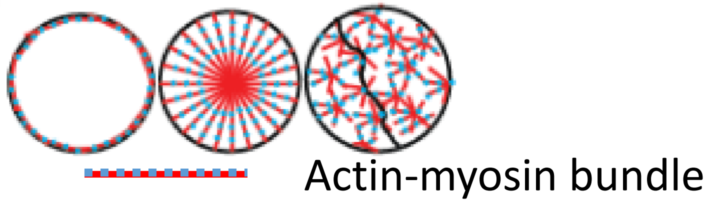


$$\frac{\dot{L}}{L_0} = -\frac{2V_h}{D} \cdot P(k, N)$$

Probability for contraction: P



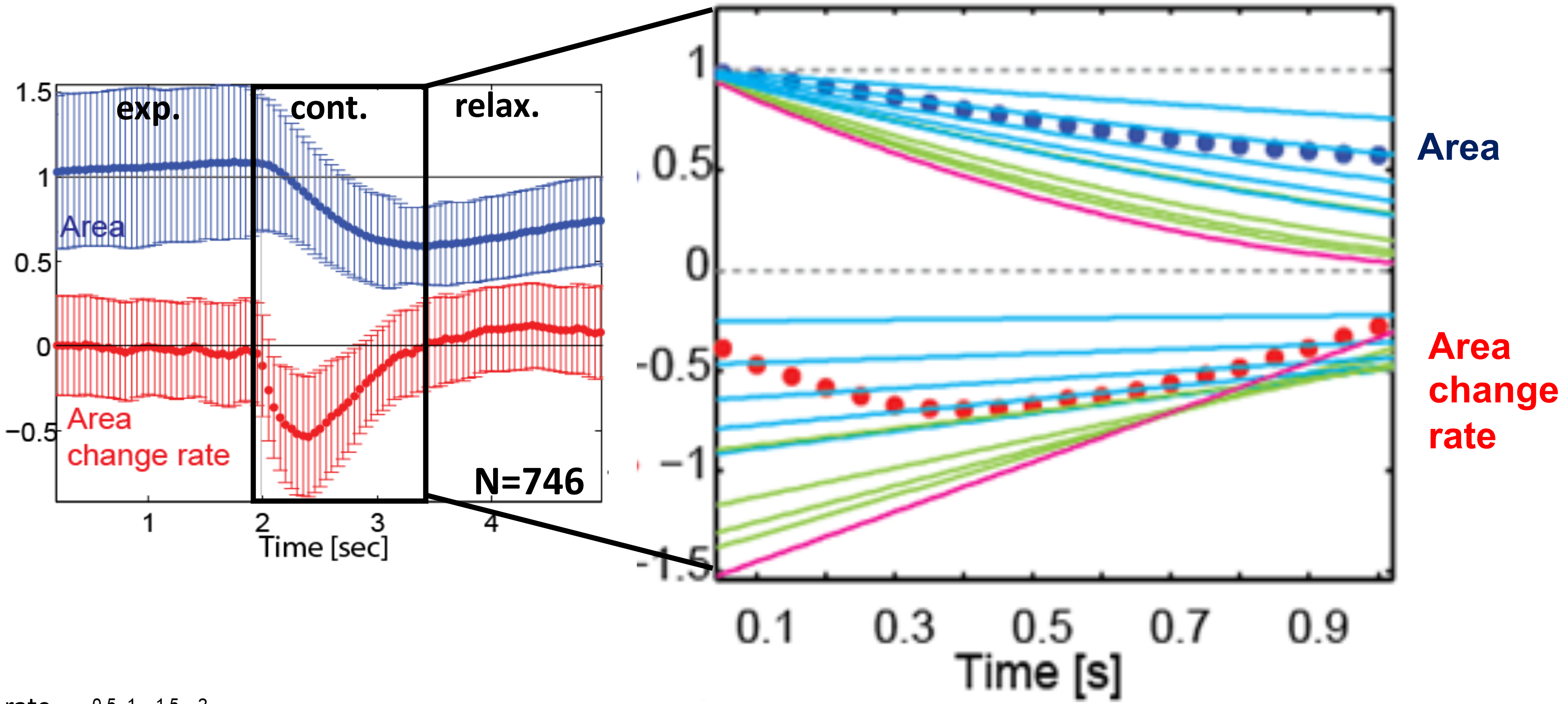
$$P = 1/6$$

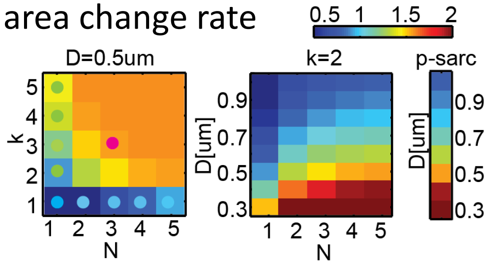


$$\dot{A} = \frac{-4V_h A_0}{D}\left(-P + \frac{V_h}{D} P^2 t\right)$$

Armon et al, PNAS 2018

Known machinery can “easily” yield these speeds in <u>free cables</u>



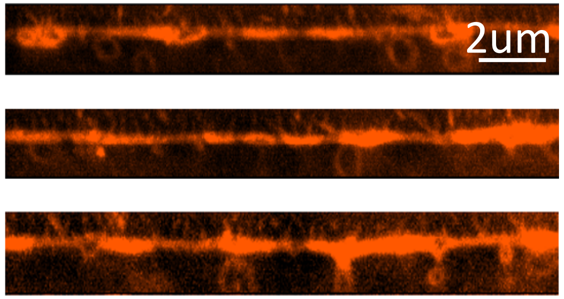


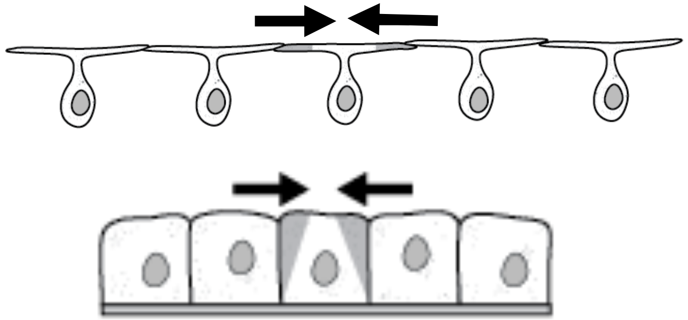
Armon et al, PNAS 2018

Why only Tplax? Tissue Minimizes Load

1) Tissue is extremely thin

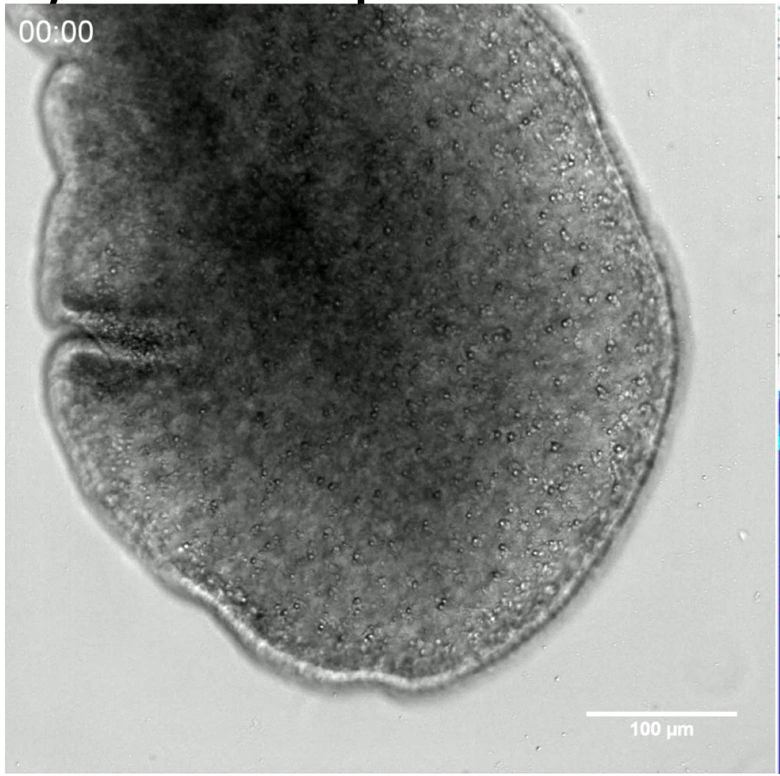
Live cross-sections

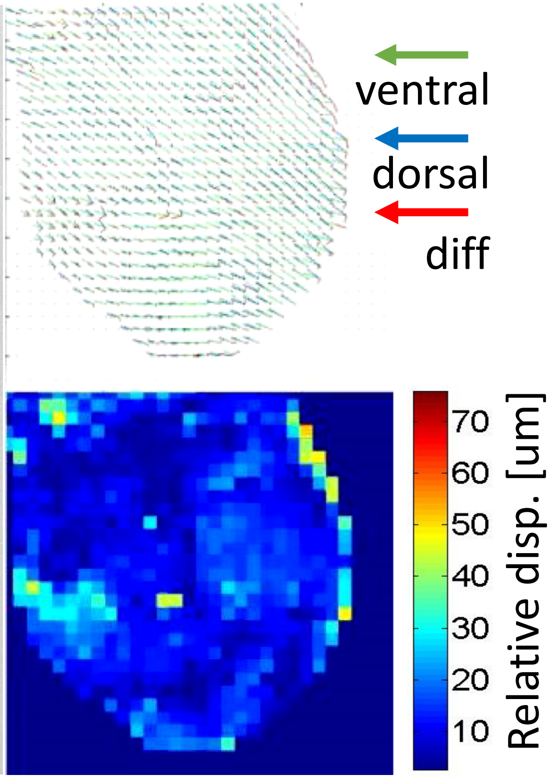




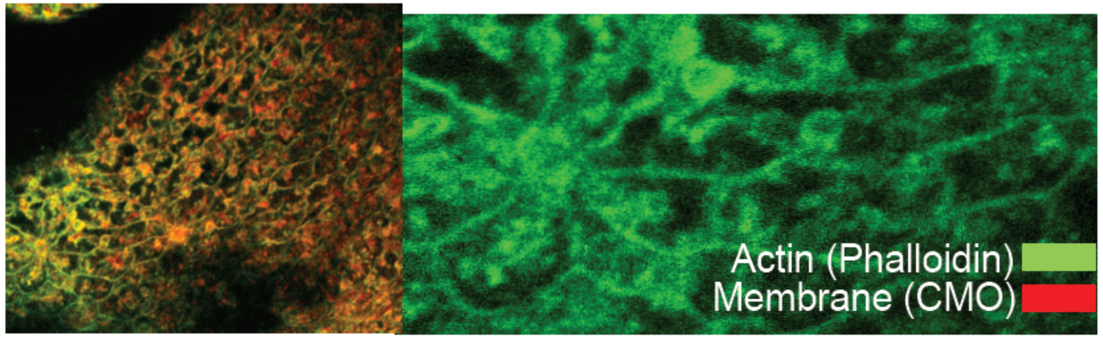
5x more force

2) Tissue is suspended



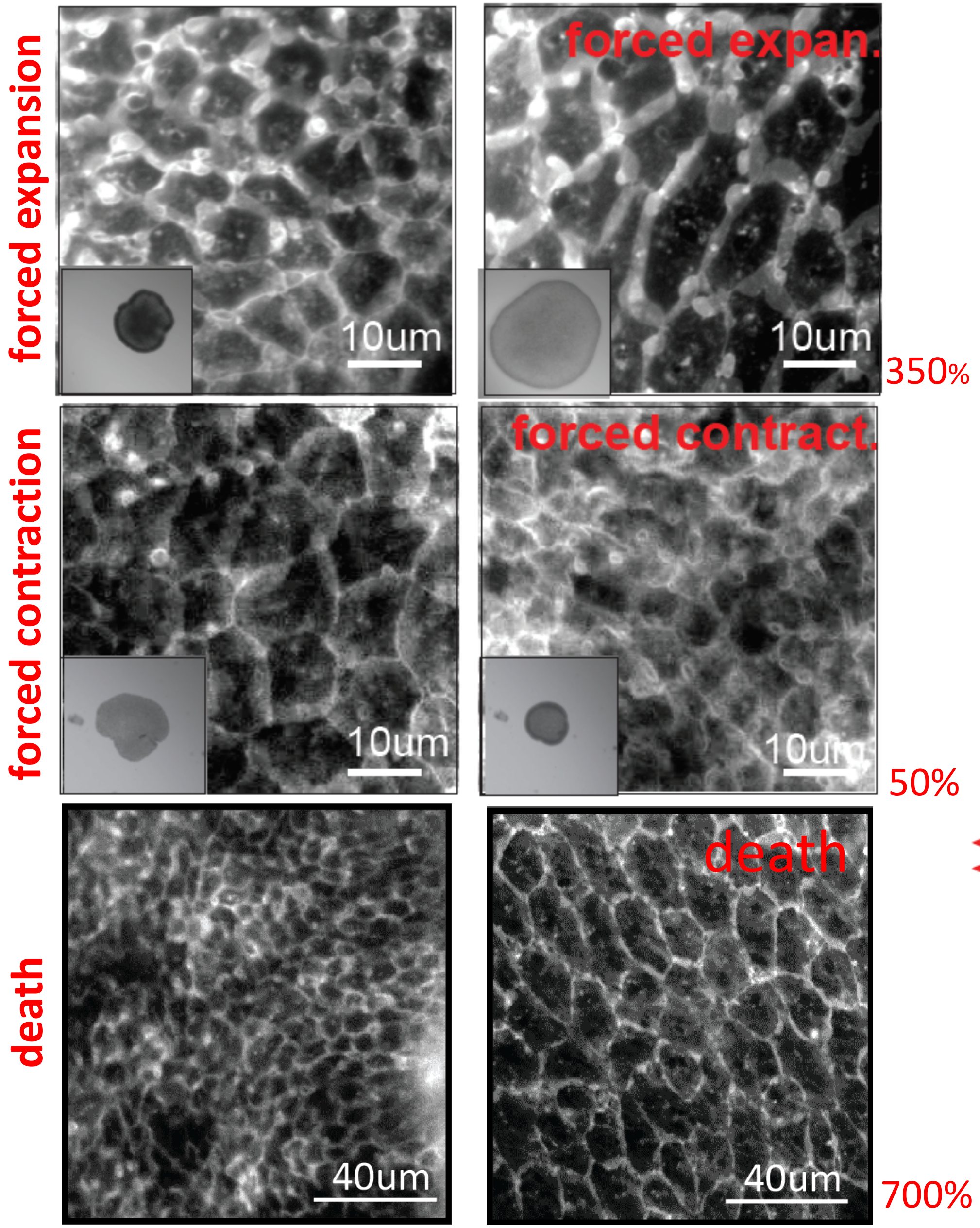


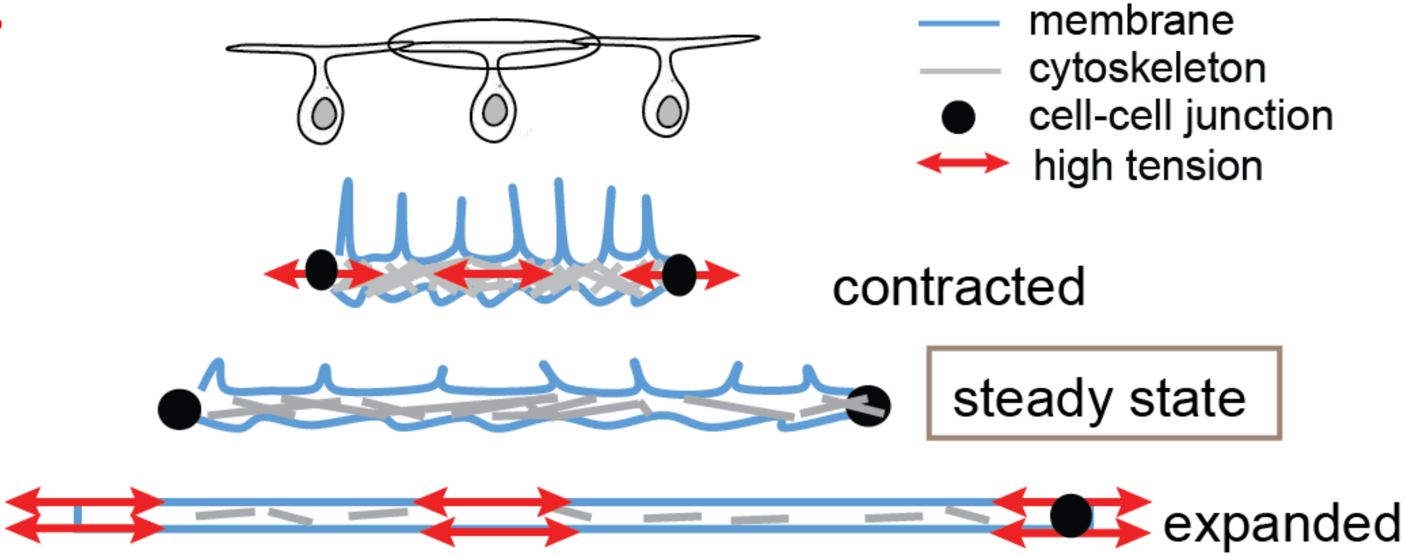
3) Peripheral actin bundles (purse-string)



Armon et al, PNAS 2018

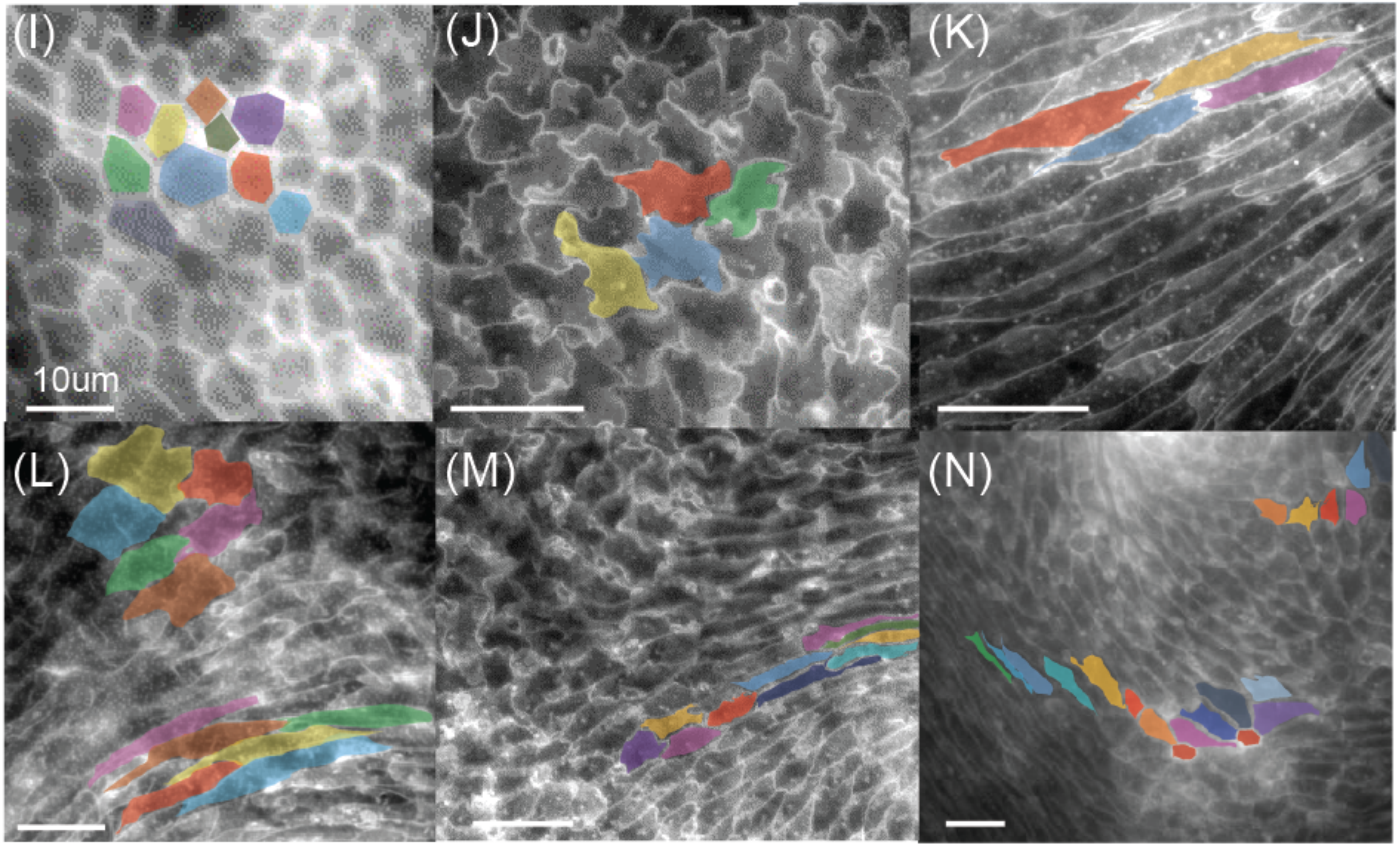
4) Cell size variation and steady state





Armon et al, PNAS 2018

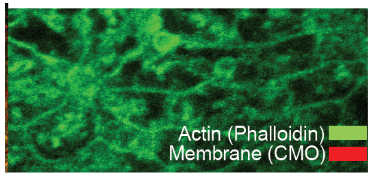
5)Cell shape and stiffness variation



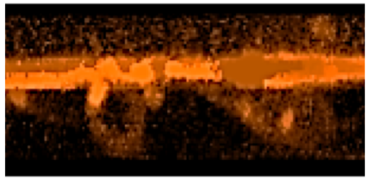
Armon et al, PNAS 2018

50% cell-area in 1 sec?!?!

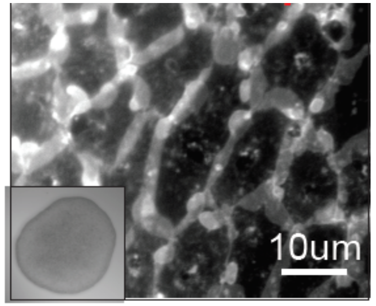
1. ActoMyosin machinery is capable

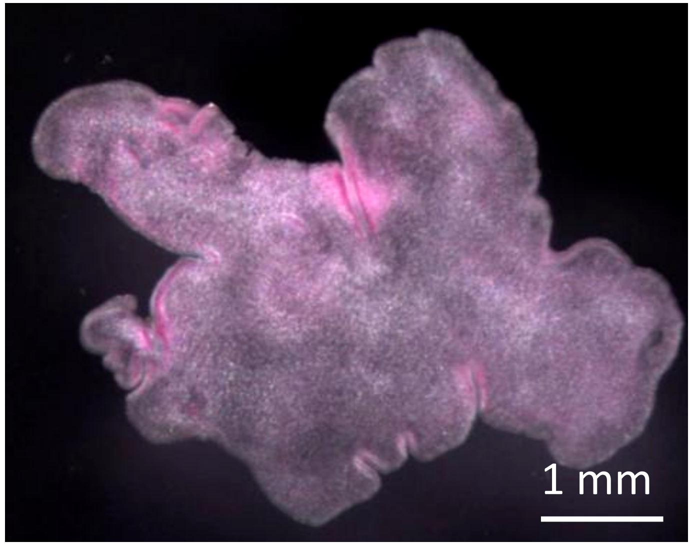


2. Architecture minimized load on contractions



3. Neighbor-cells are ready to yield





Contraction Dynamics in *Trichoplax Adhaerens*

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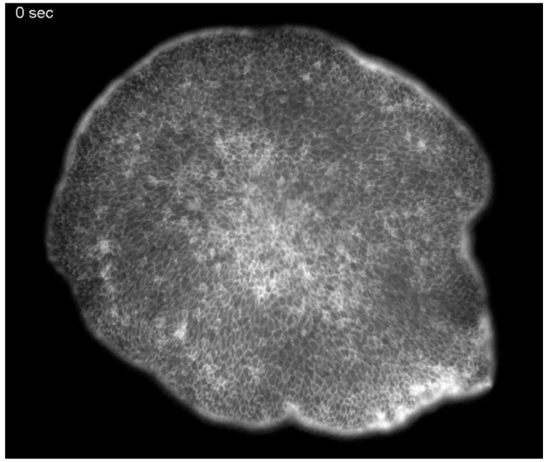
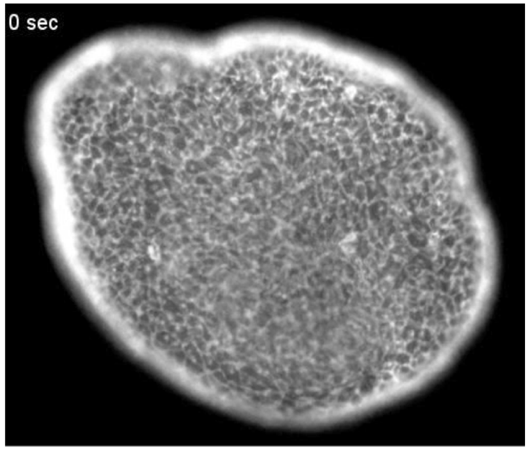
3. Story #2: Tissue dynamics
   and the "active cohesion" hypothesis

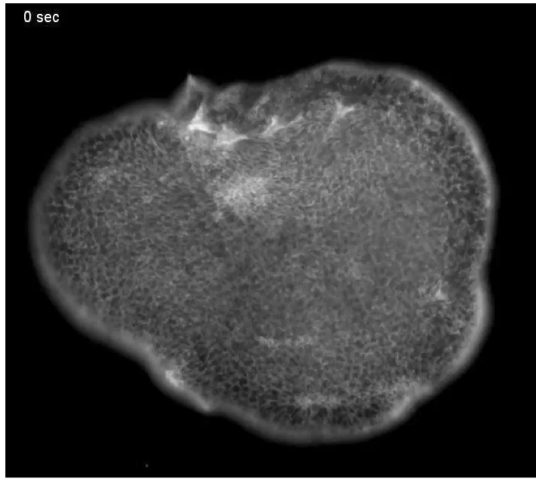
sparse



radial



uni-axial



biological process	time scale/cell [sec]
Protein translation	25
Actin turnover	20
Tplax radial waves	1.5
Tplax uniaxial waves	0.3
Diffusion	10^{-1}
Viscoelasticity	10^{-2}-10^{-3}
Neuronal transmission	10^{-7}

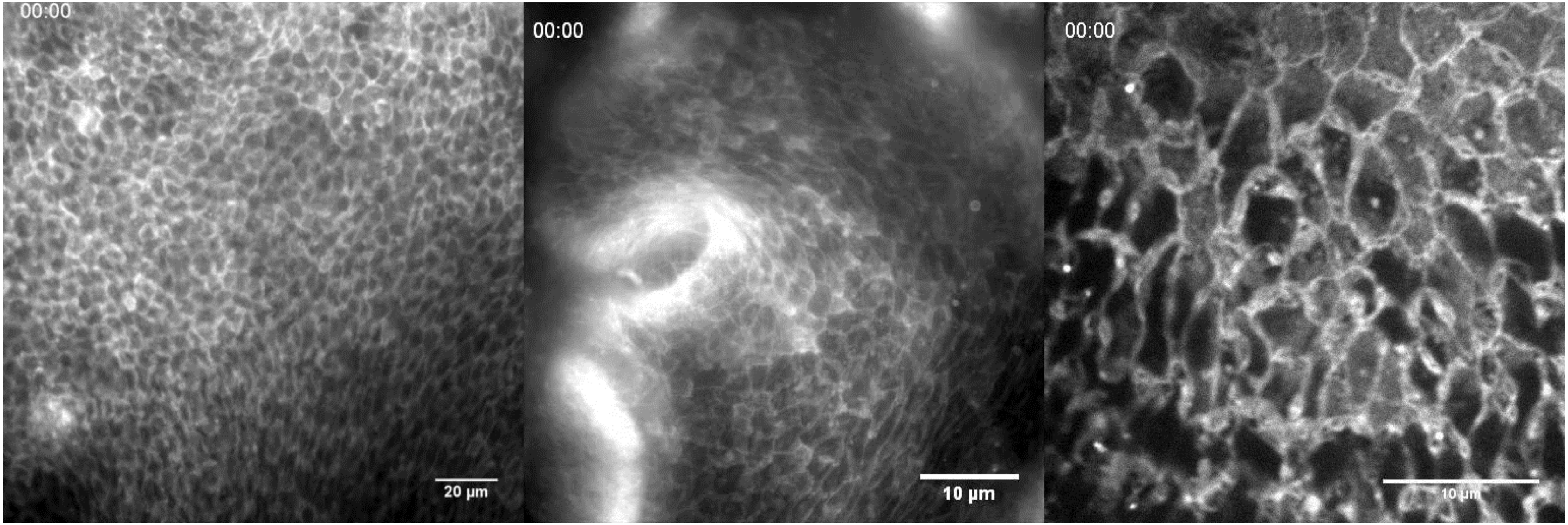
slow

fast

(#1) Is mechanics involved in wave propagation?

Armon et al, PNAS 2018

The miserable life of a cell:



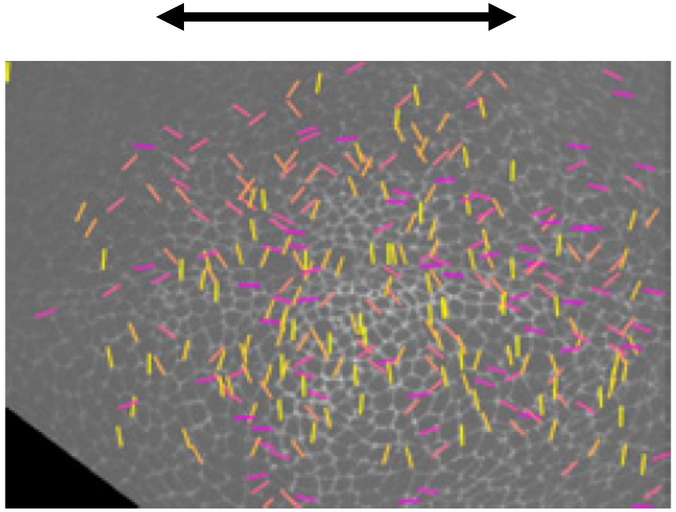
Despite alternating stresses, and quick changes in cells' size and shape,
The tissue always stays intact.

(#2) How is integrity maintained?

Tissue response to tensile stress:

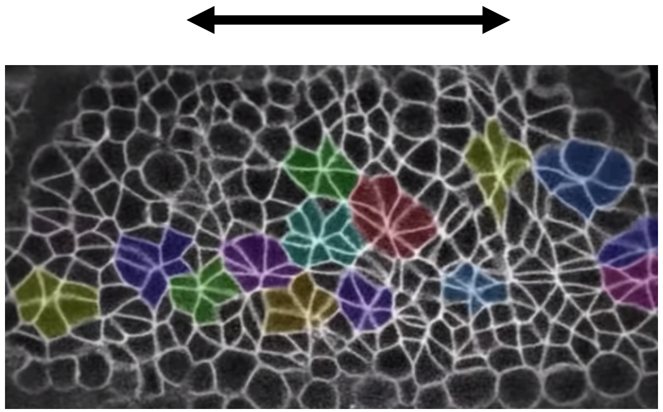
1. Oriented cell divisions

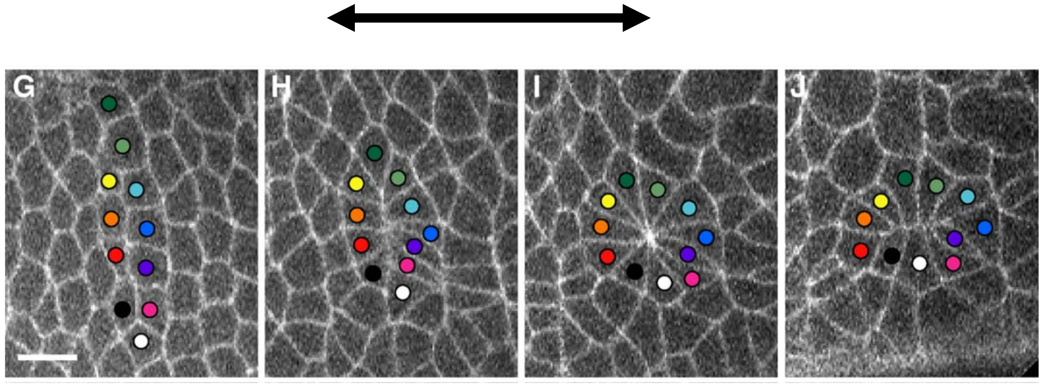
Spindle orientations



Zhou et al. Curr.Bio. 2019

2. Cell flows

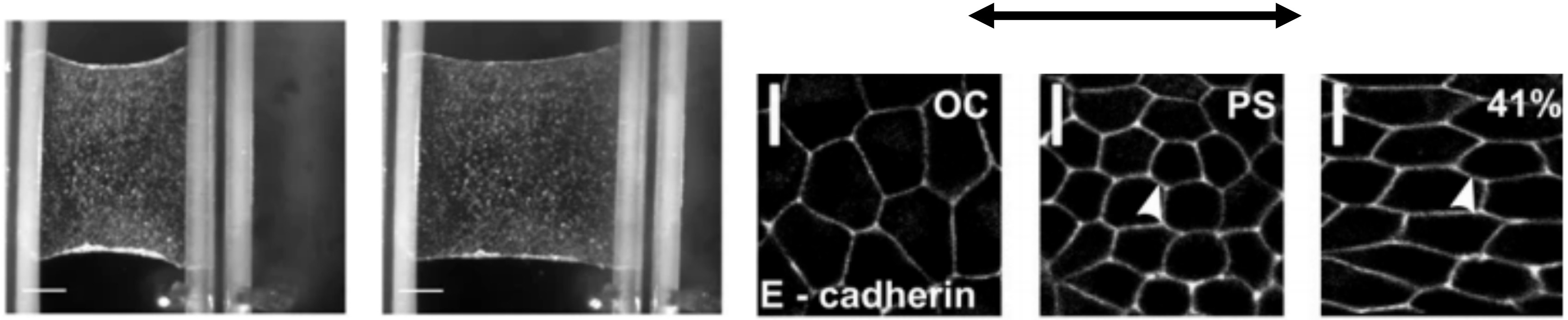




Blankenship et al, Developmental cell 2006

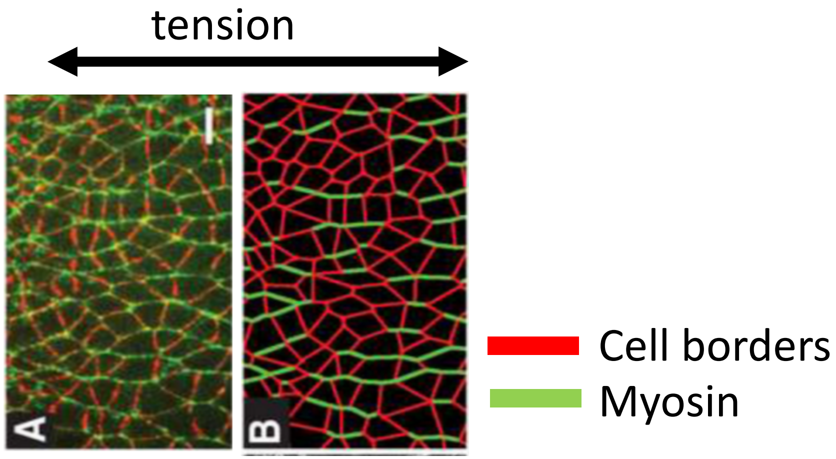
Tissue response to tensile stress:

3. Active softening

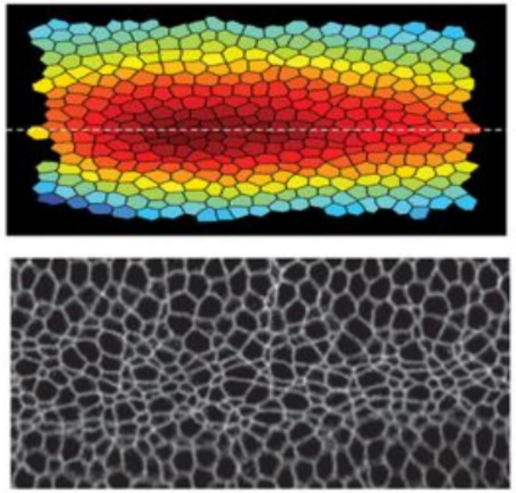


Khalilgharibi et al., Nature Physics 2019.

4. Active contraction:

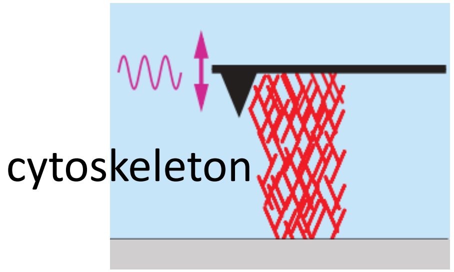


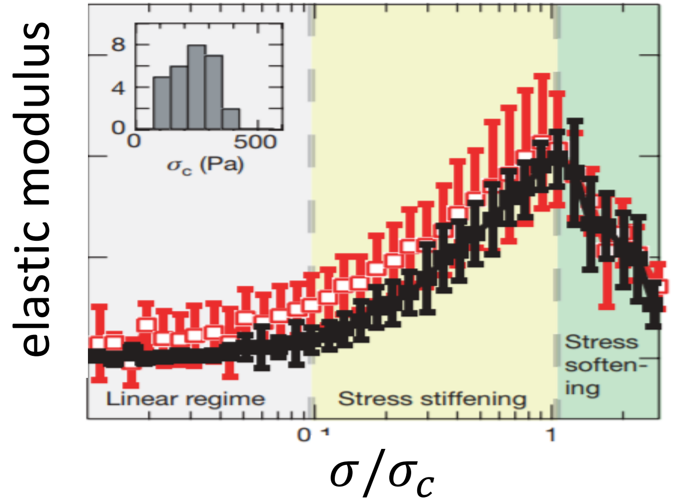
Fernandez-Gonzales Dev Cell 2009



Noll et al Nature Physics 2017

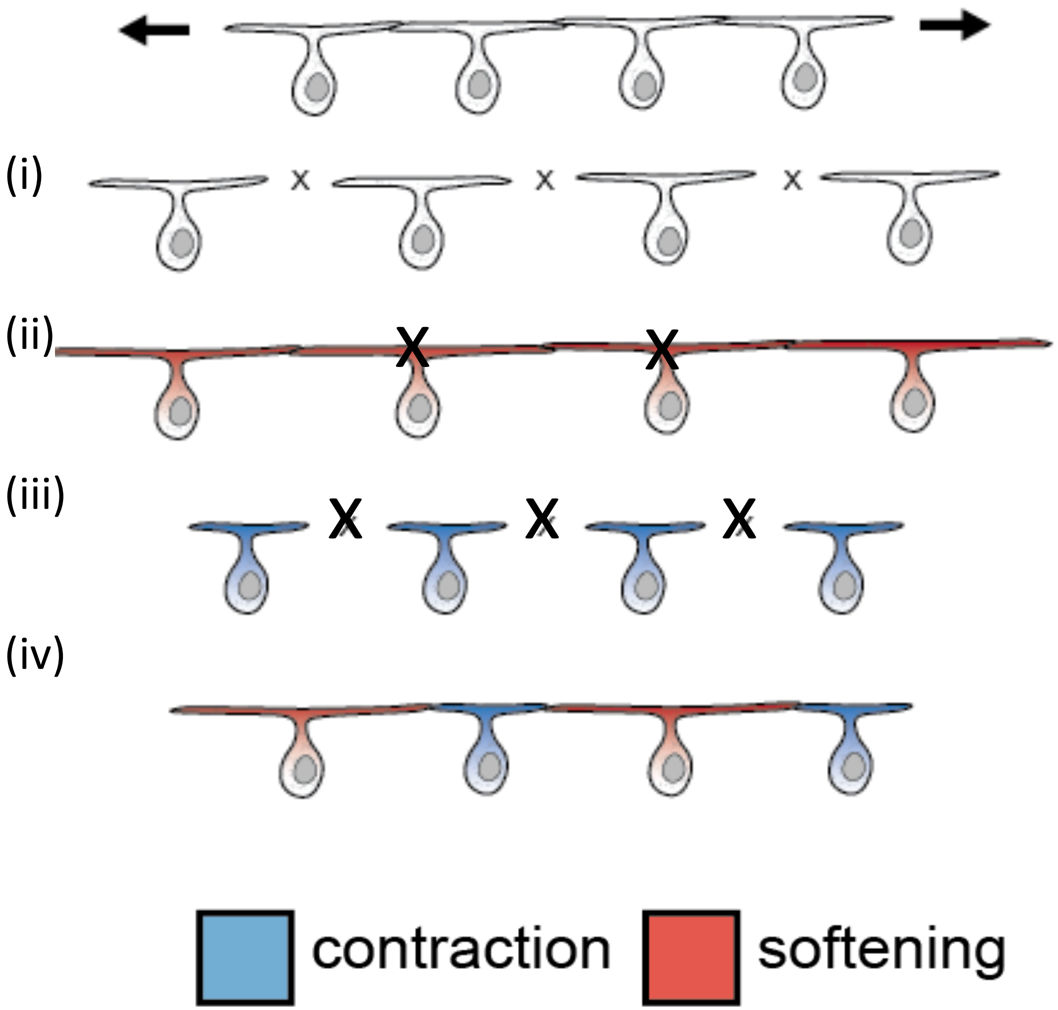
Molecular-level experiments show **BOTH** stress stiffening and stress softening:





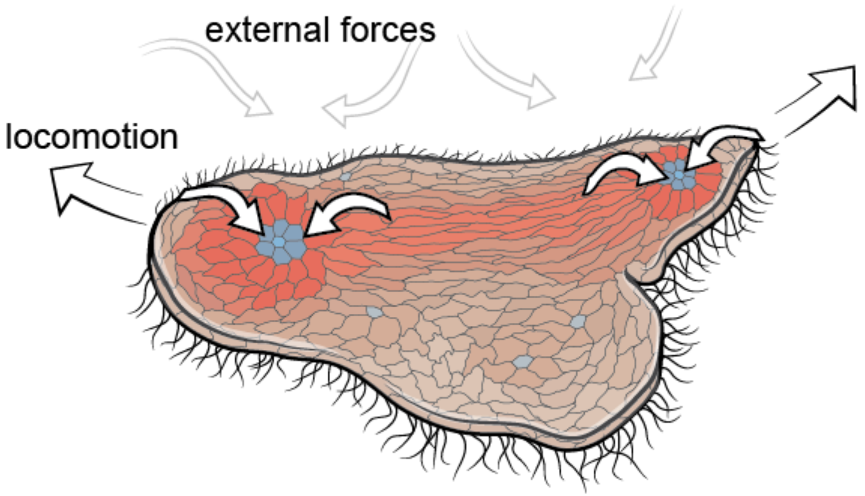
Chaudhuri et al, Nature 2007

Tissue has two failure modes:



Contraction + softening =

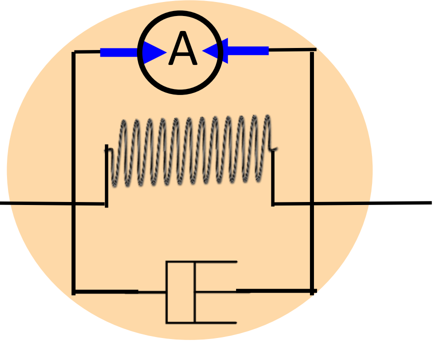
"Active Cohesion" ?



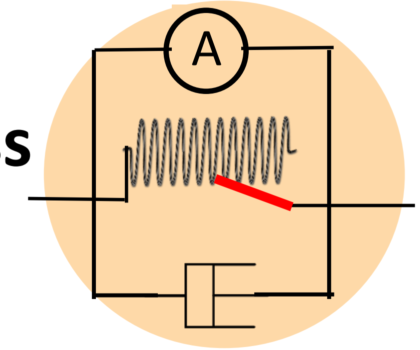
Modeling the two switches

Single cell

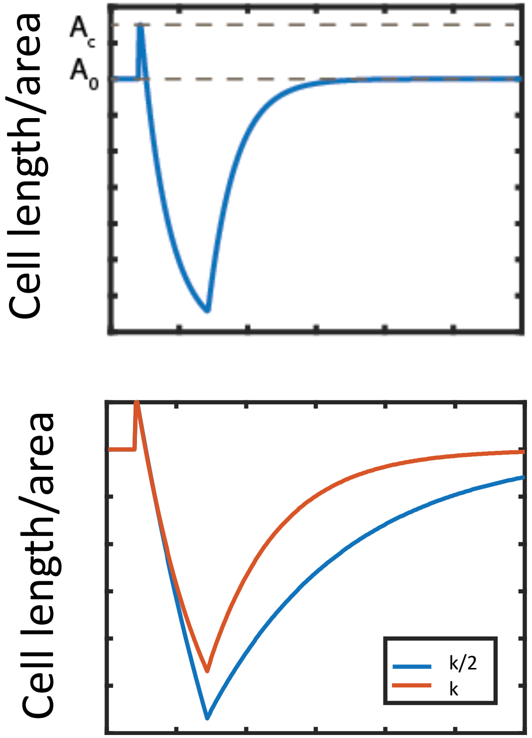
(i)
Avoiding high cell **strain**
By active **cell contraction**:



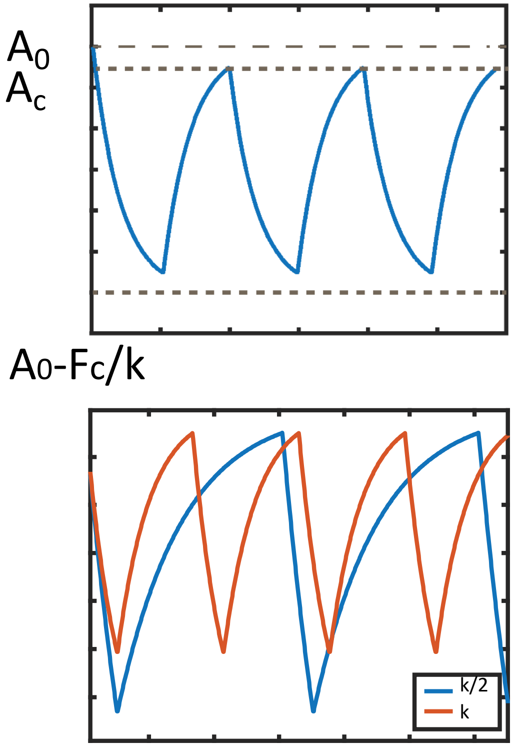
(ii)
Avoiding high **junction stress**
By local **cell softening**:



excitable mode



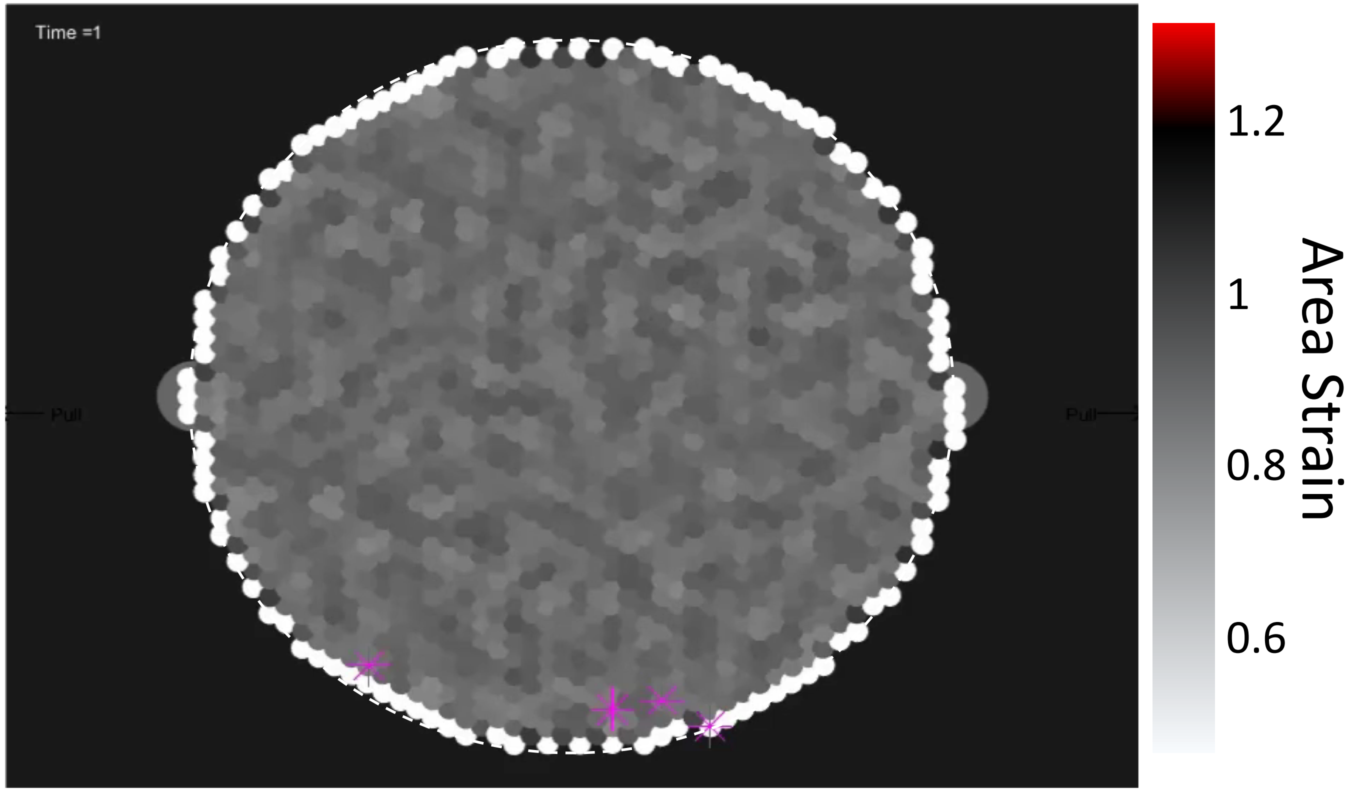
relaxation oscillator



Modeling the two switches

(i)
Avoiding high **cell strain**
By active **cell contraction**:

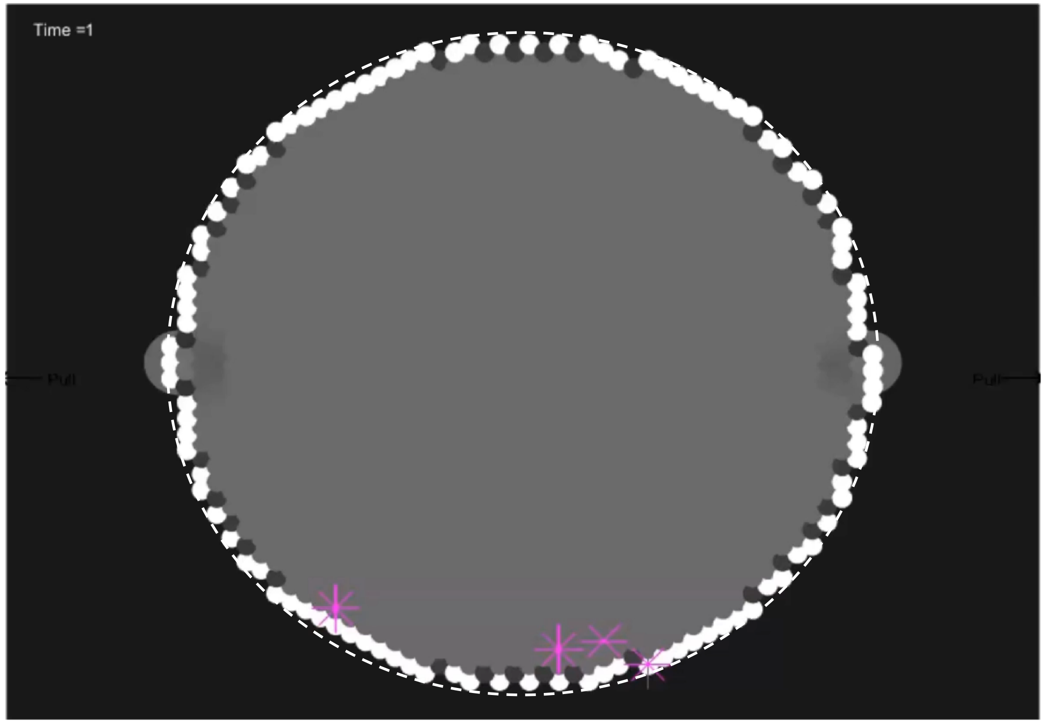
(ii)
Avoiding high **junction stress**
By local **cell softening**:



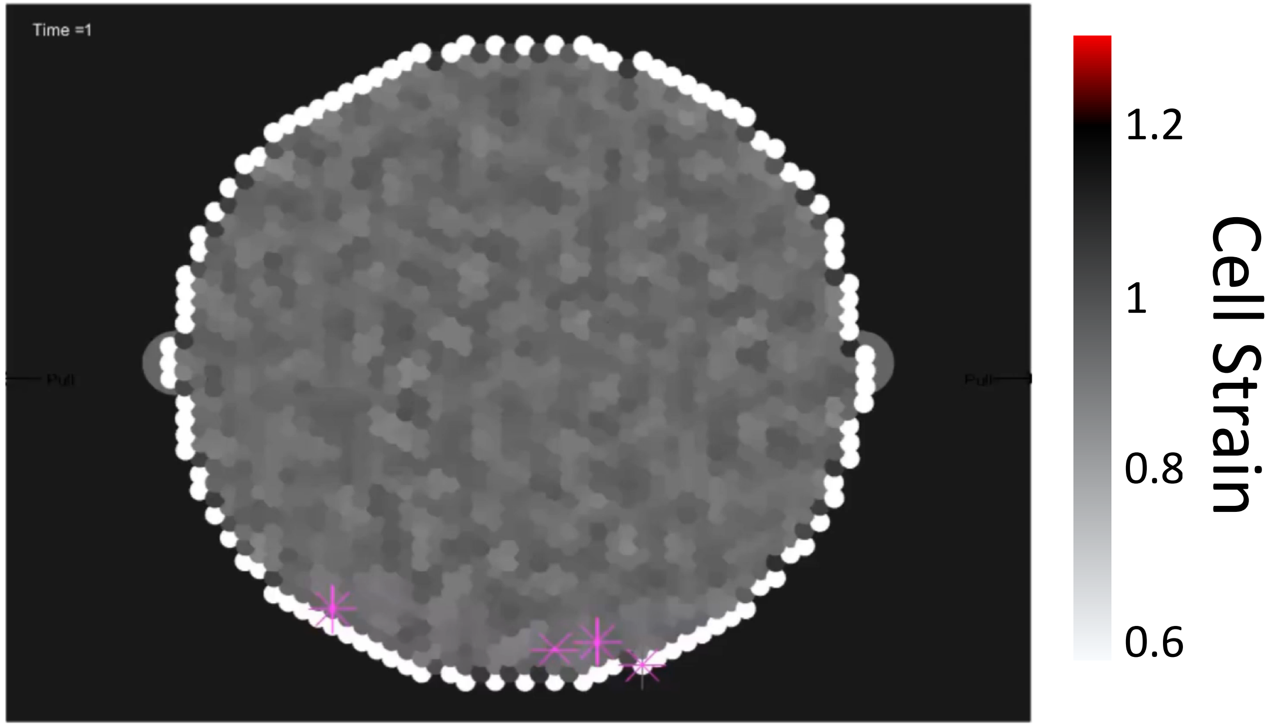
Matt Bull

Response to stretch:

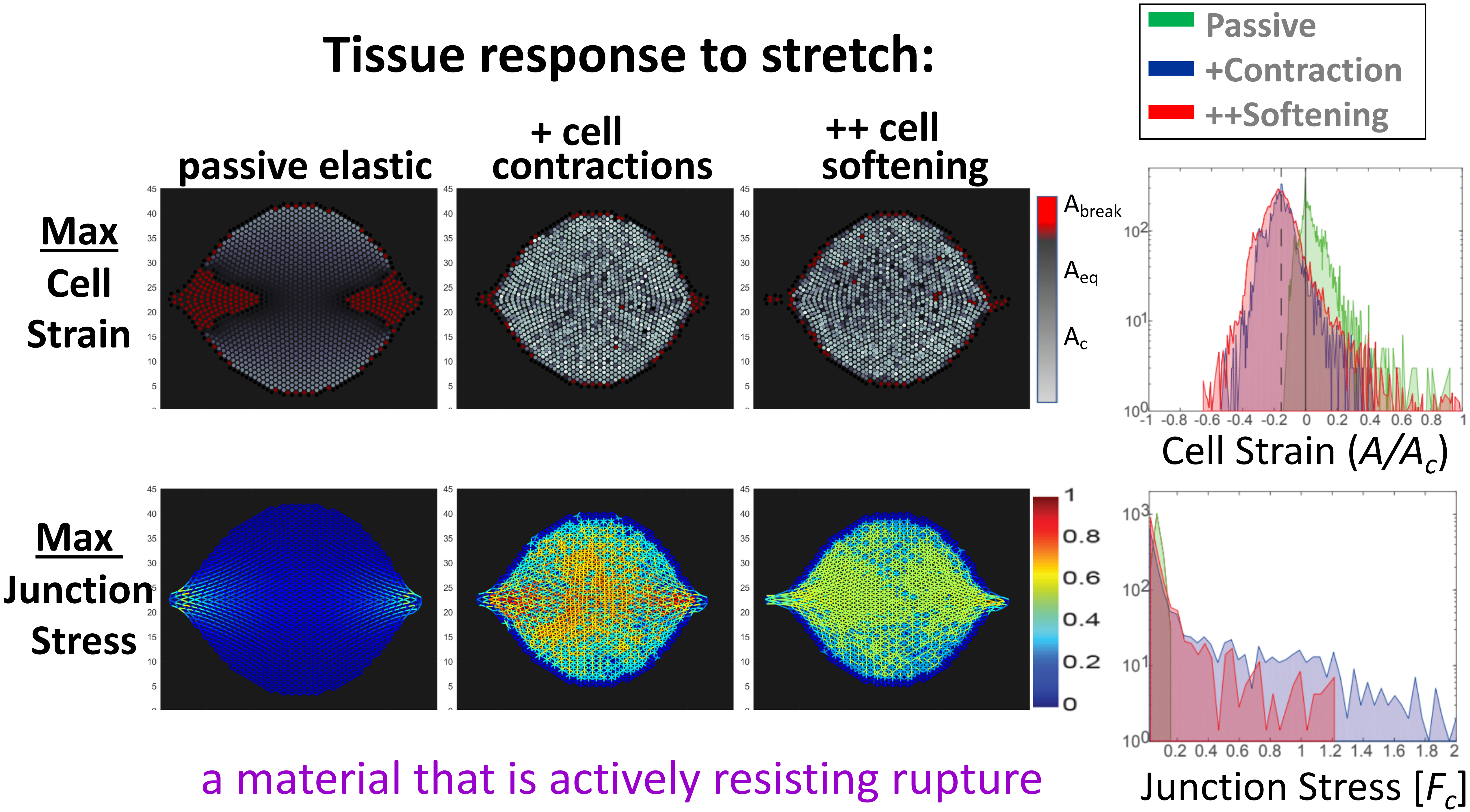
Passive elastic



+contractions



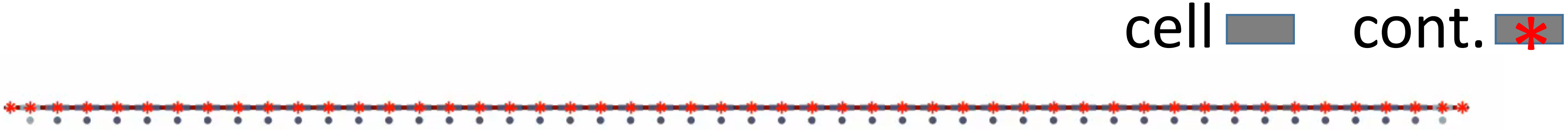
Tissue response to stretch:



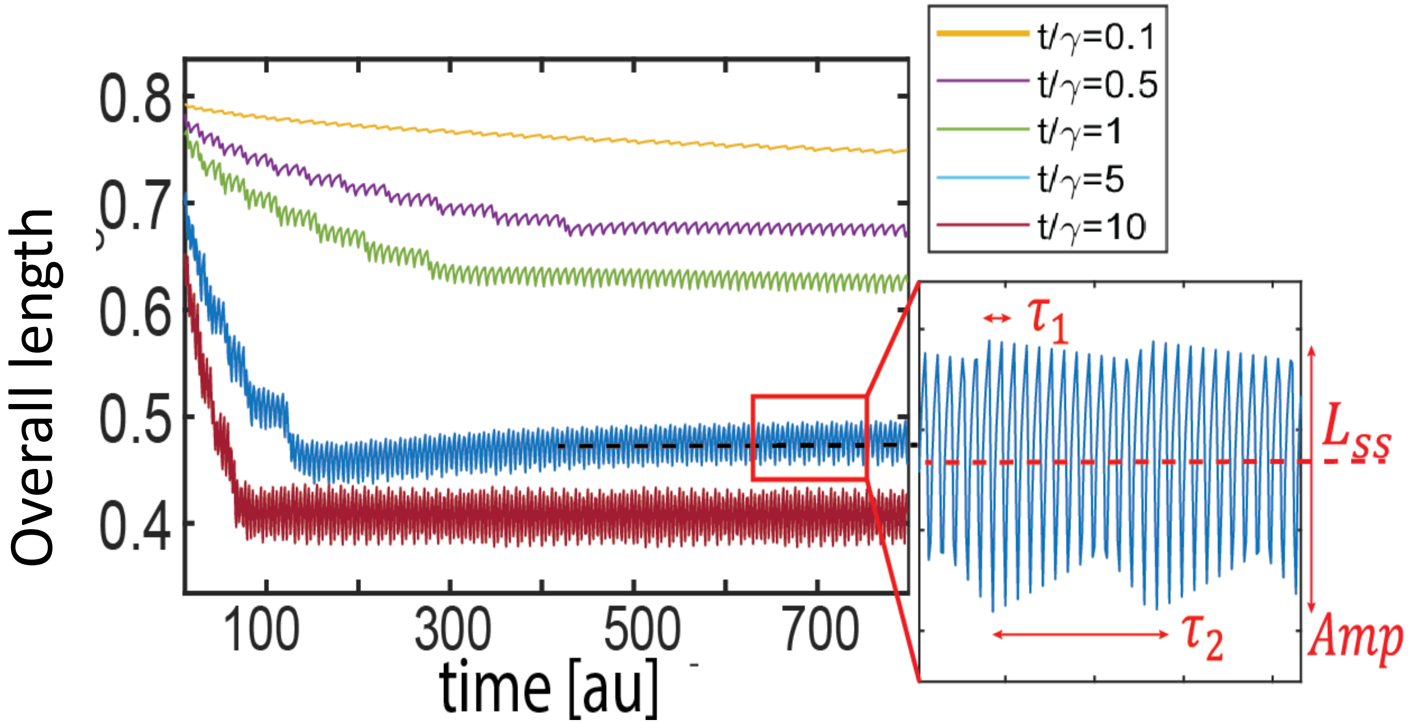
a material that is actively resisting rupture

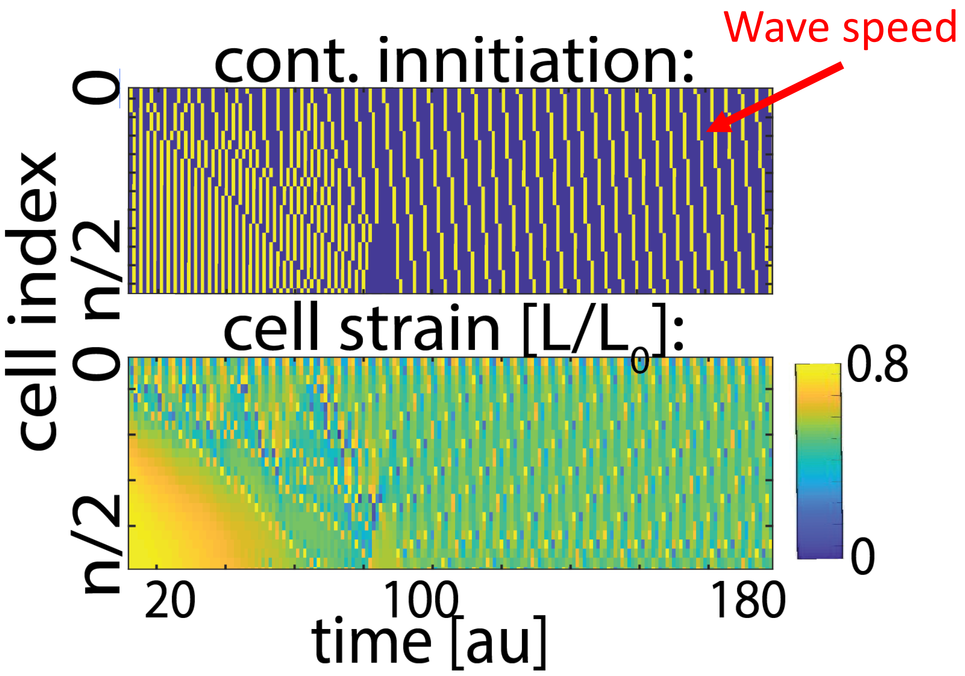
1D simulation:

Isolated Waves

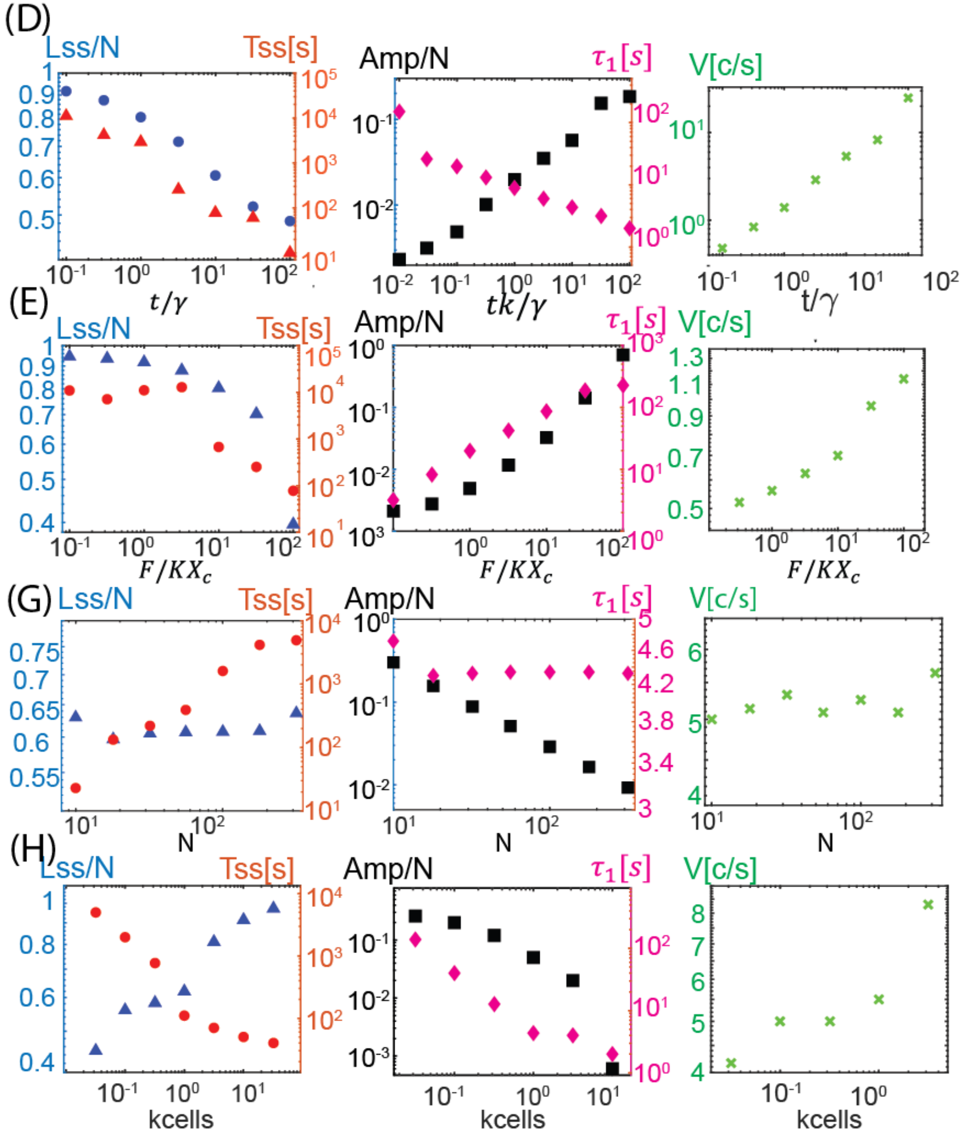


measurables:





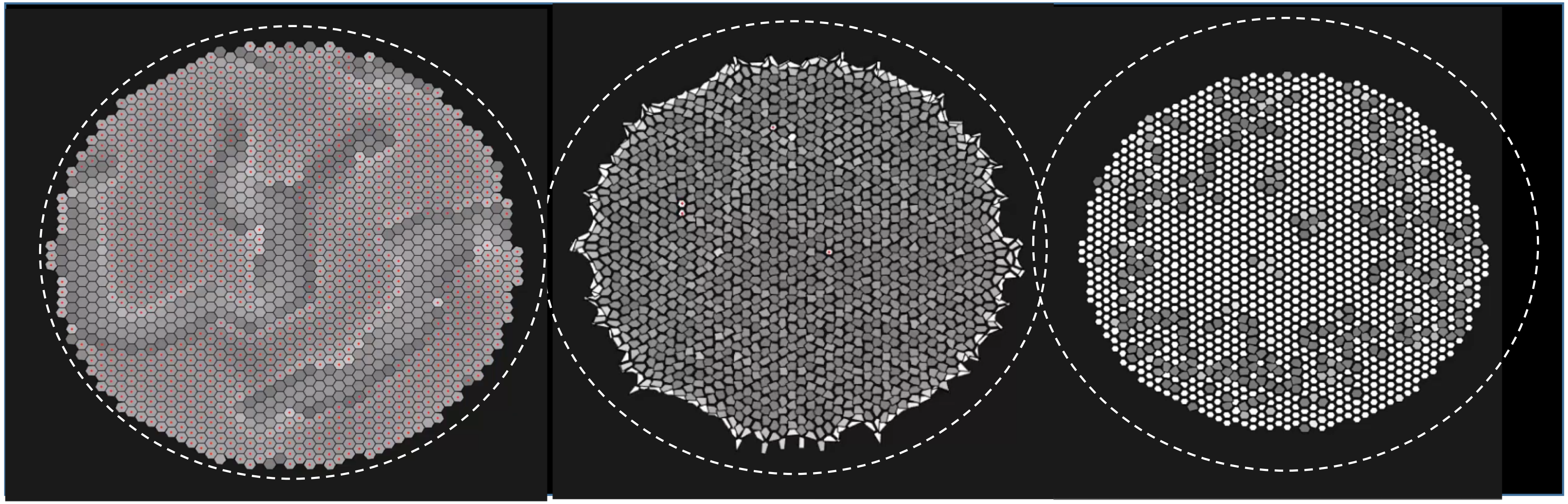
A lot of data



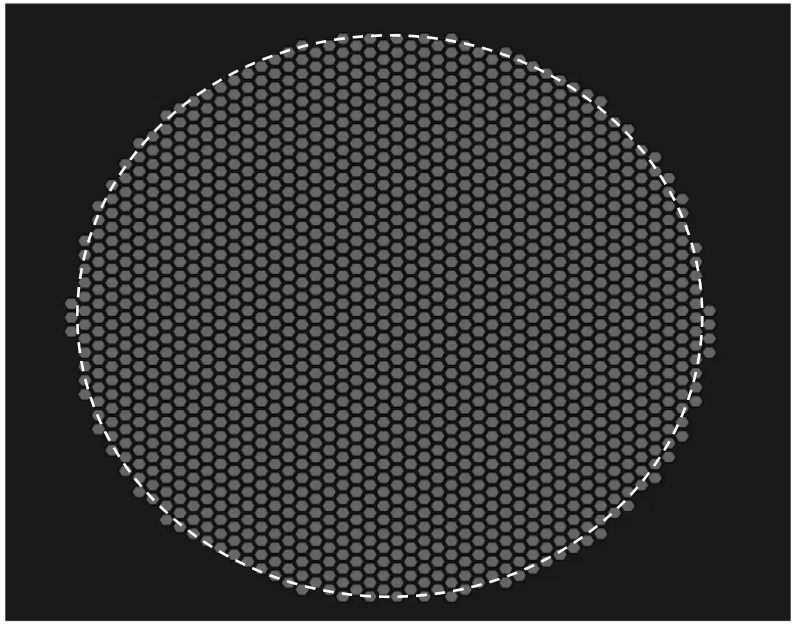
Contraction Waves (in 1D)

1. Waves propagate via the viscosity of the media.
2. Spontaneous waves propagate from the rim inwards, in a non-constant speed (slower in the bulk).
3. Noise can create waves anywhere (but slower in the bulk).
4. Waves are non linear and annihilate.
5. Stiffer cells make waves go faster.

Continuous waves | Long quiescence | Inverse quiescence 50-50



Excitable +pinch



Unique to 2D:

Long quiescence

- Residual stresses
- distorted shapes

Active cohesion – future directions

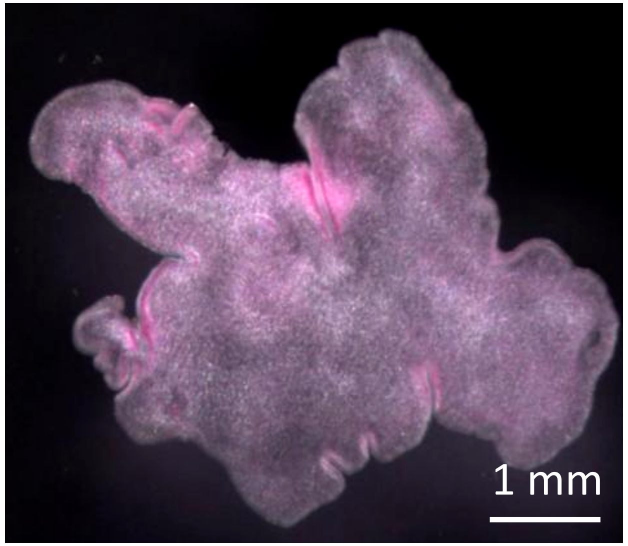
Theory and experiment
Other tissue types (embryonic or not)

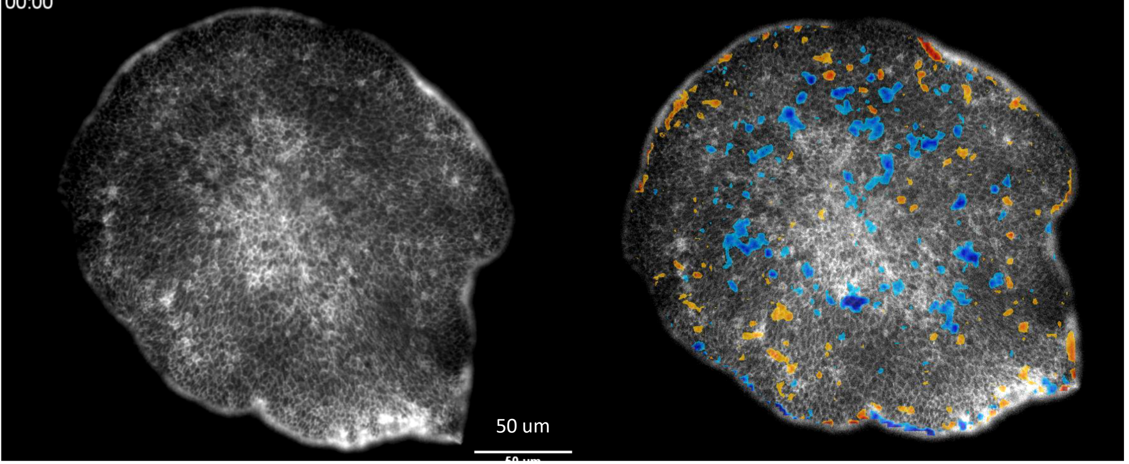
Tplax as a model system for epithelium biomechanics

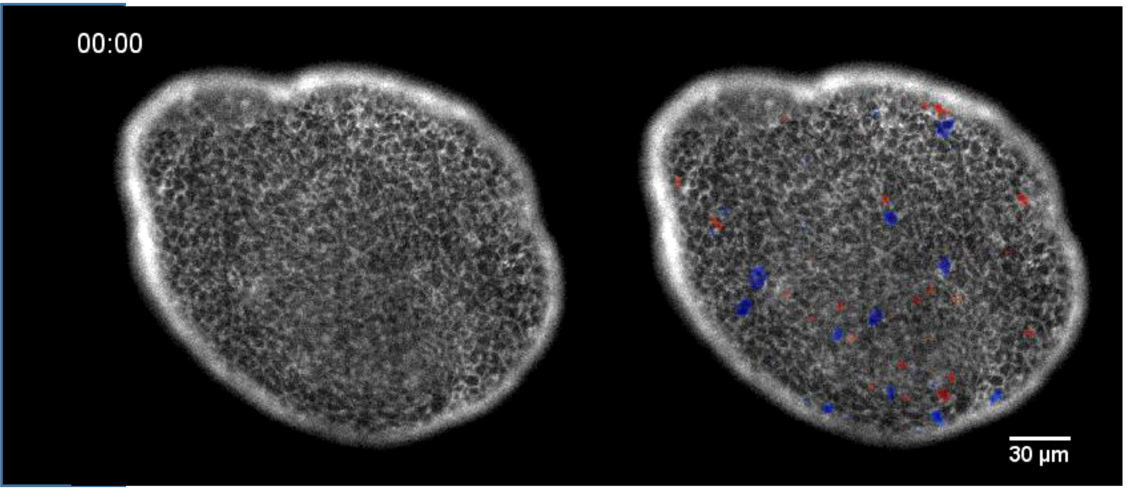
- **2D animal:** imaging, manipulation, modeling
- **Minimalism:** short genome, 6 cell types, no ECM/BM, only adherens junctions
- **Speed of events** – faster than genetic and biochemical time scales.

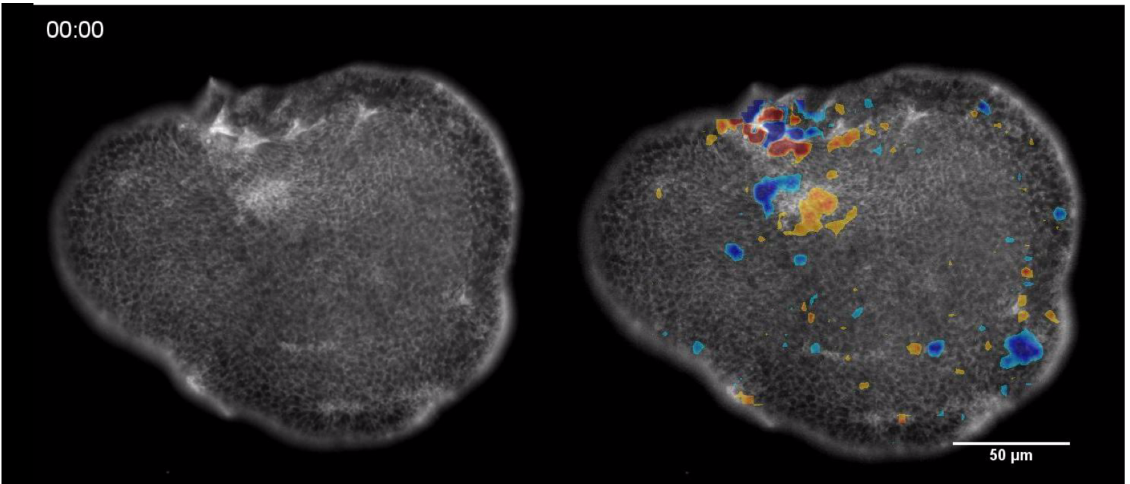
Mechanics must be sensed and activated

- **High strains** – stresses can be "seen"







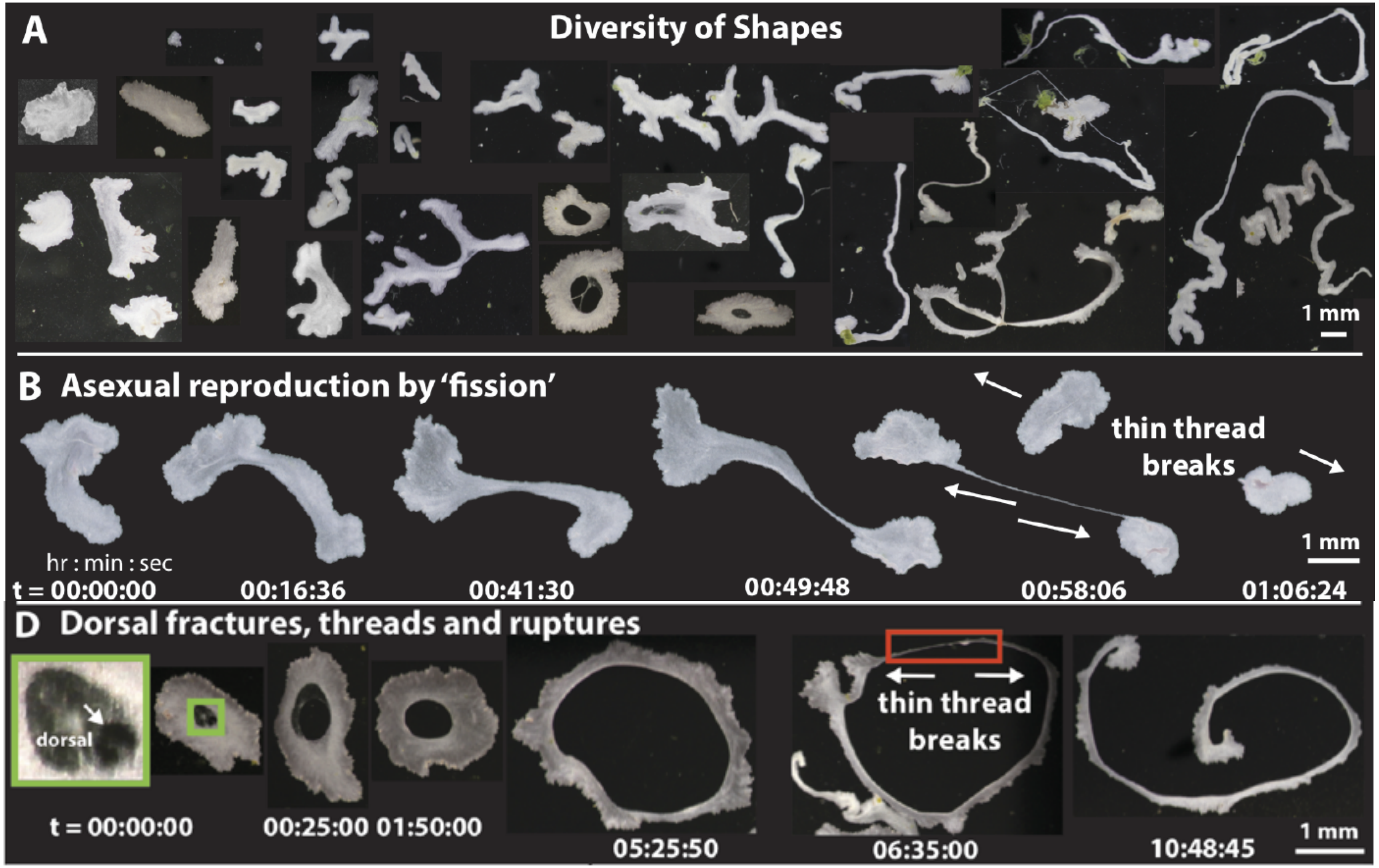


Thanks!

Manu Prakash

Matt Bull

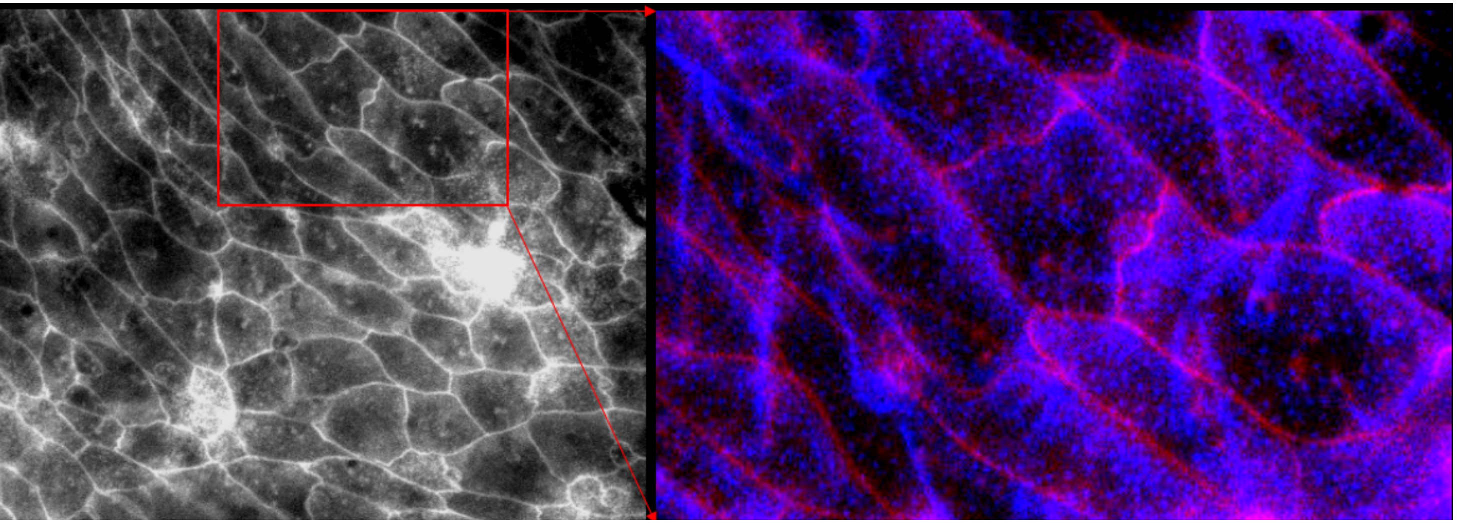
Andres Aranda-Diaz   Deepak Krishnamurthy   Vivek Prakash

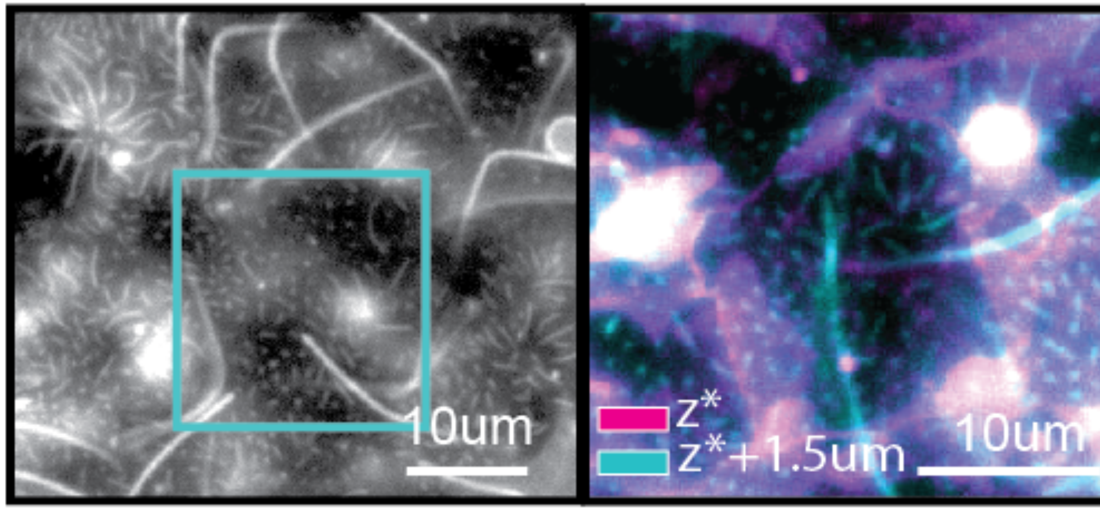


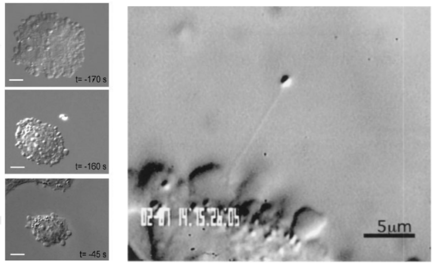
V. Prakash et al, bio-arxiv 2019

Membrane tubes

Live membrane stain

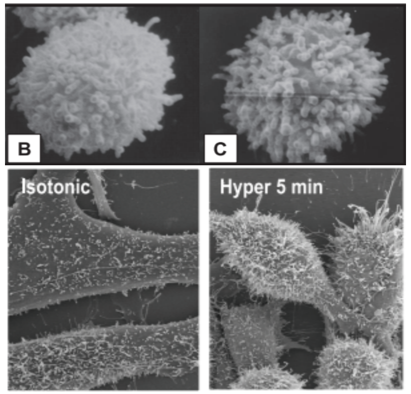






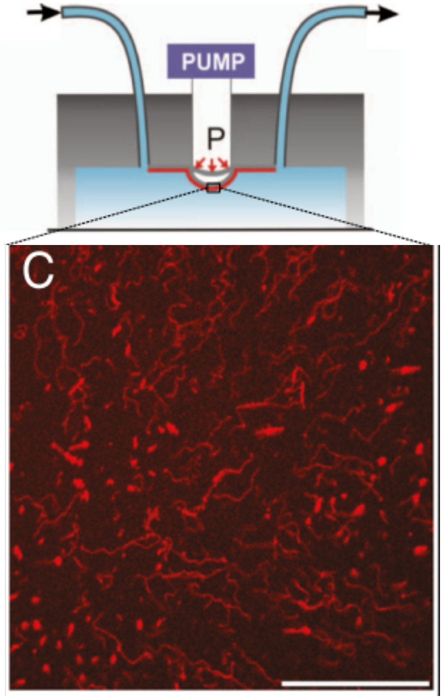
Charras Nature 2005

hyper-pressure:
blebbing

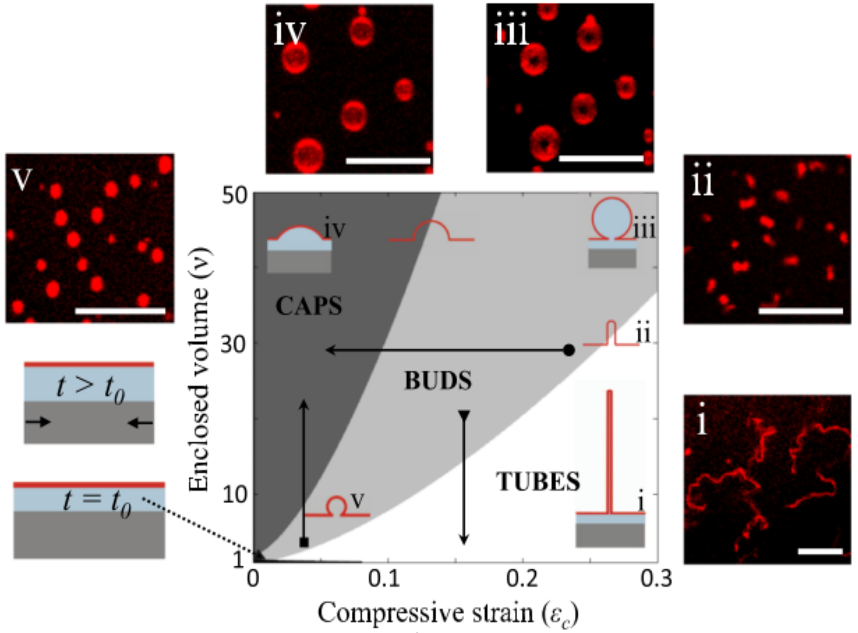


Hoffman, Physiol.Rev, 2009

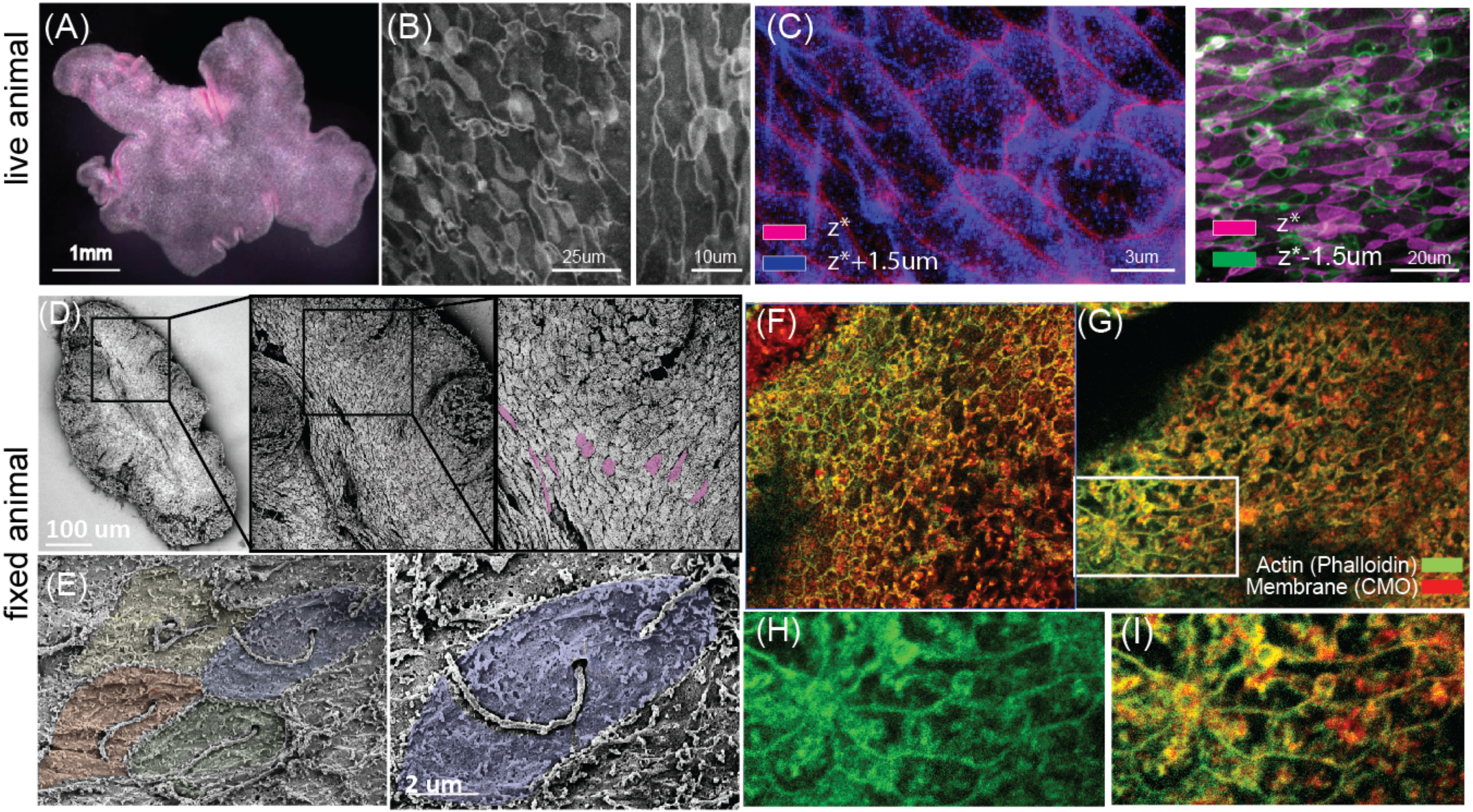
hipo-pressure:
Membrane tubes



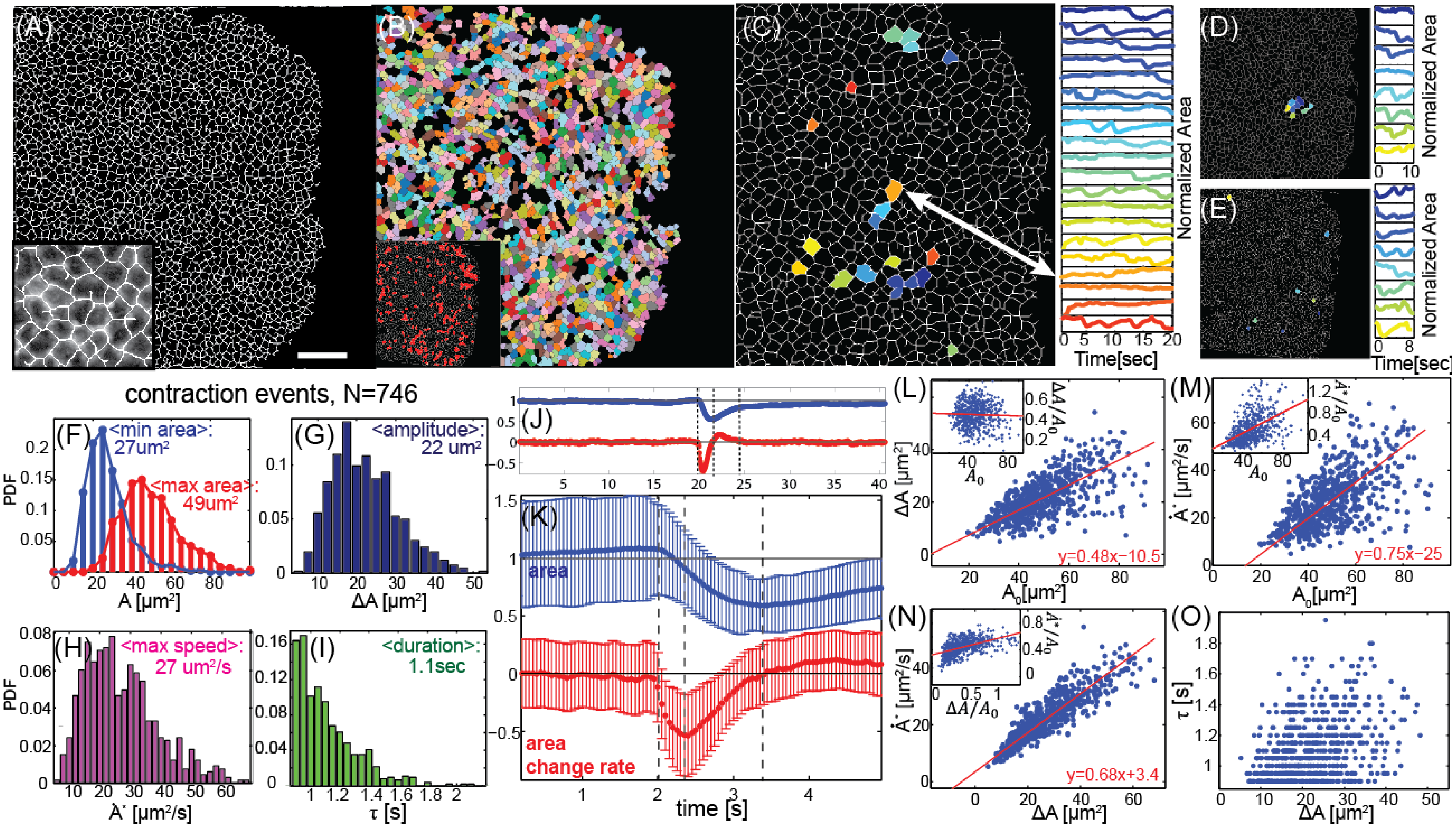
M. Staykova PNAS 2011

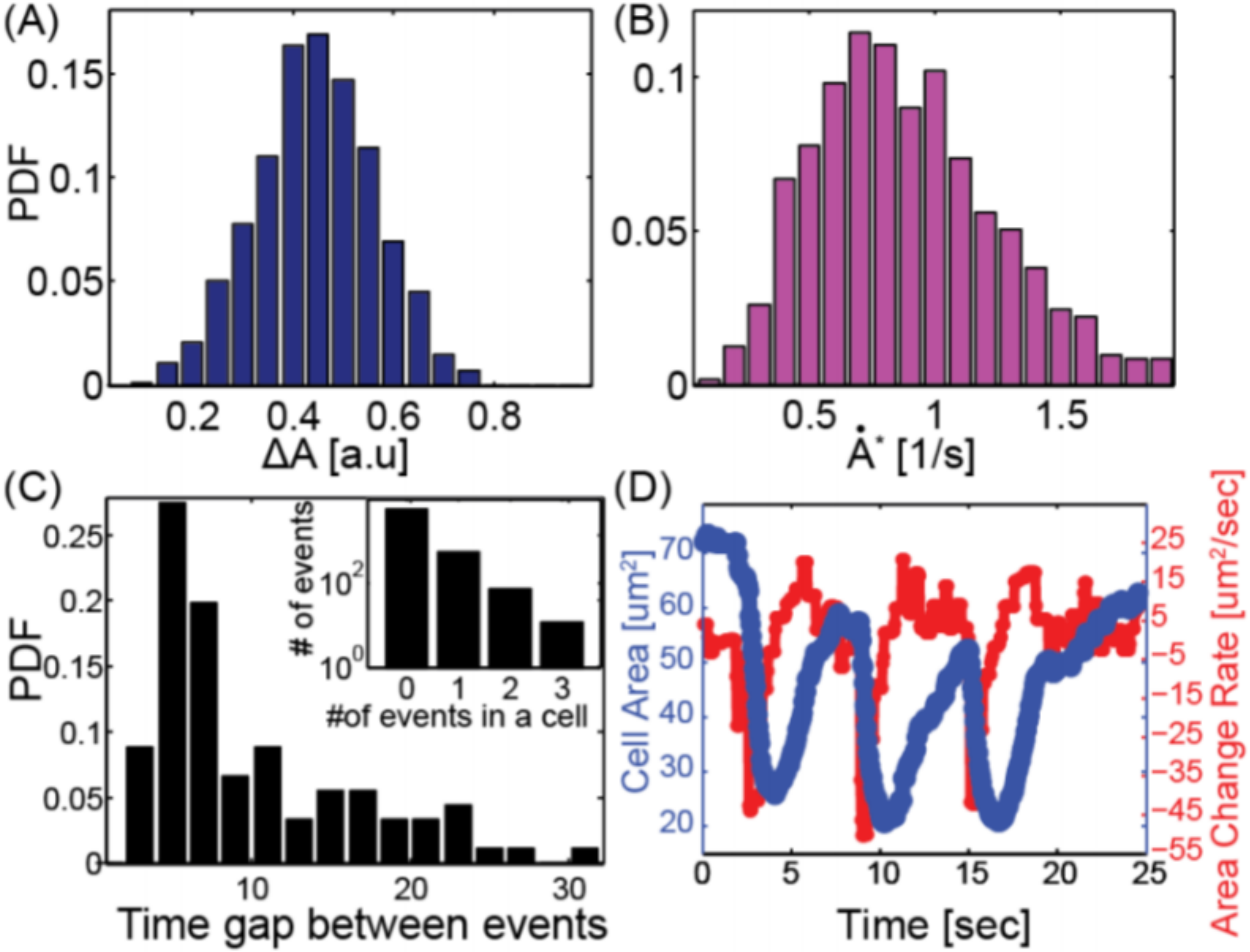


M. Staykova PRL 2013

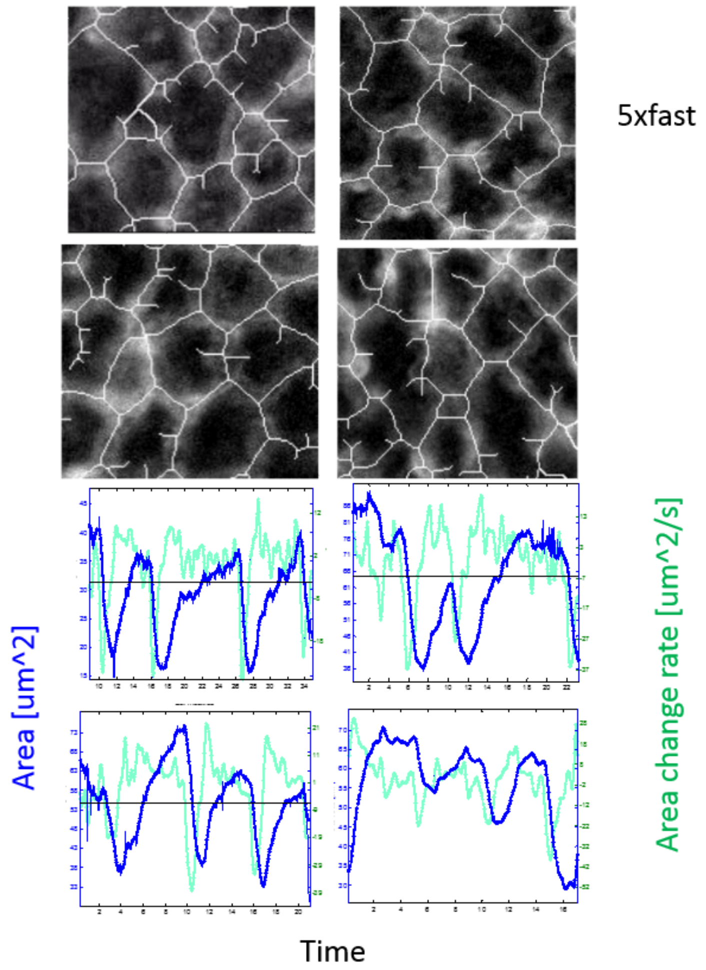


live animal
fixed animal
(A)
1mm
(B)
25um
10um
(C)
z*
z*+1.5um
3um
z*
z*-1.5um
20um
(D)
100 um
(E)
2 um
(F)
(G)
Actin (Phalloidin)
Membrane (CMO)
(H)
(I)


(A)
(B)
(C)
Normalized Area
Time[sec]
(D)
(E)
contraction events, N=746
(F)
<min area>: 27um²
<max area>: 49um²
PDF
A [µm²]
(G)
<amplitude>: 22 um²
ΔA [µm²]
(H)
<max speed>: 27 um²/s
Ȧ* [µm²/s]
(I)
<duration>: 1.1sec
τ [s]
(J)
(K)
area
area change rate
time [s]
(L)
ΔA/A0
A0
ΔA [µm²]
A0[µm²]
y=0.48x−10.5
(M)
Ȧ*/A0
Ȧ* [µm²/s]
y=0.75x−25
(N)
ΔA/A0
y=0.68x+3.4
(O)
τ [s]



(A)
PDF
ΔA [a.u]
(B)
Ȧ* [1/s]
(C)
PDF
Time gap between events
# of events
#of events in a cell
(D)
Cell Area [um²]
Area Change Rate [um²/sec]
Time [sec]



5xfast
Area [um^2]
Area change rate [um^2/s]
Time

Stiffness matters, and emerge