Introduction to cellular biophysics

Artur Ruppel

My background

- Electrical Engineering in TU Darmstadt in Germany
- Biomedical Engineering in INP Grenoble
- PhD in Mechanobiology in Grenoble
- Now PostDoc in François Fagotto’s lab

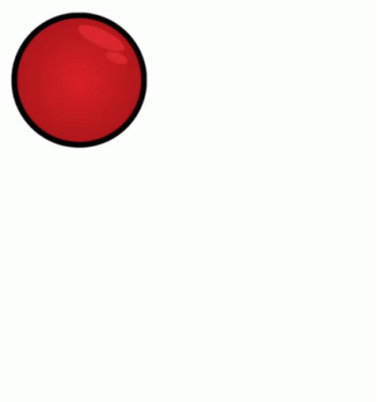
What is biophysics?

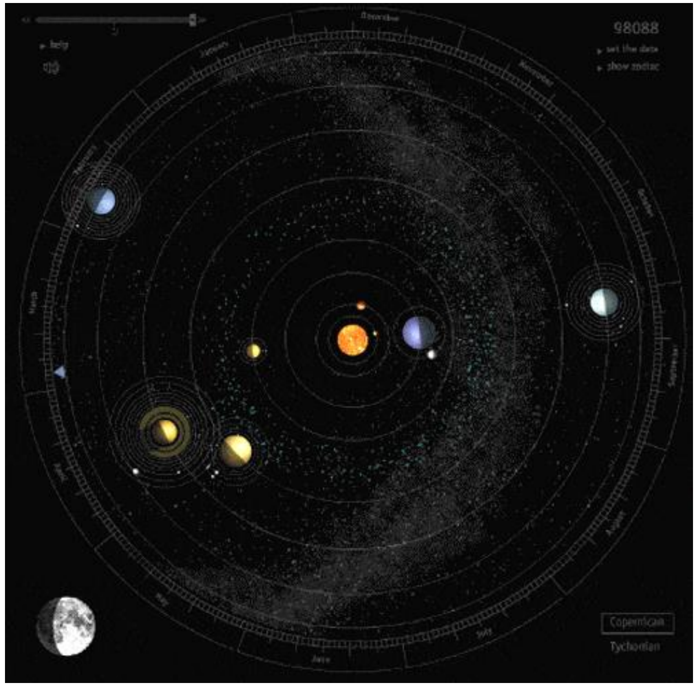
→The application of tools and traditions typically used in physics to biological phenomena

- →In particular this involves the use of mathematical models

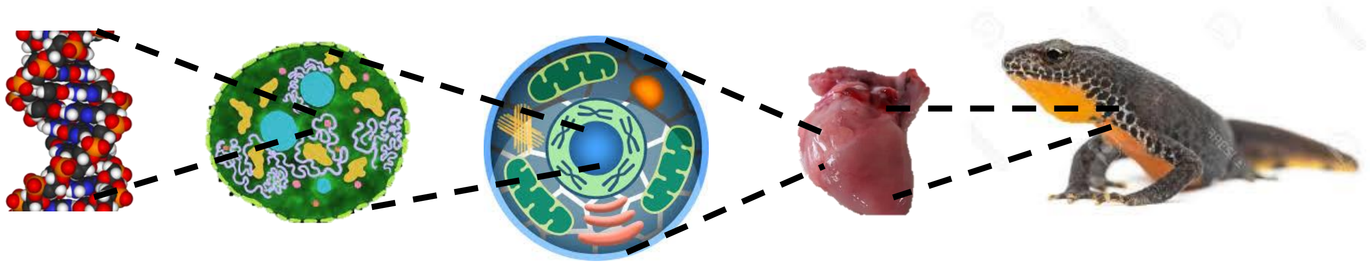
Why is maths everywhere in physics but rare in biology?

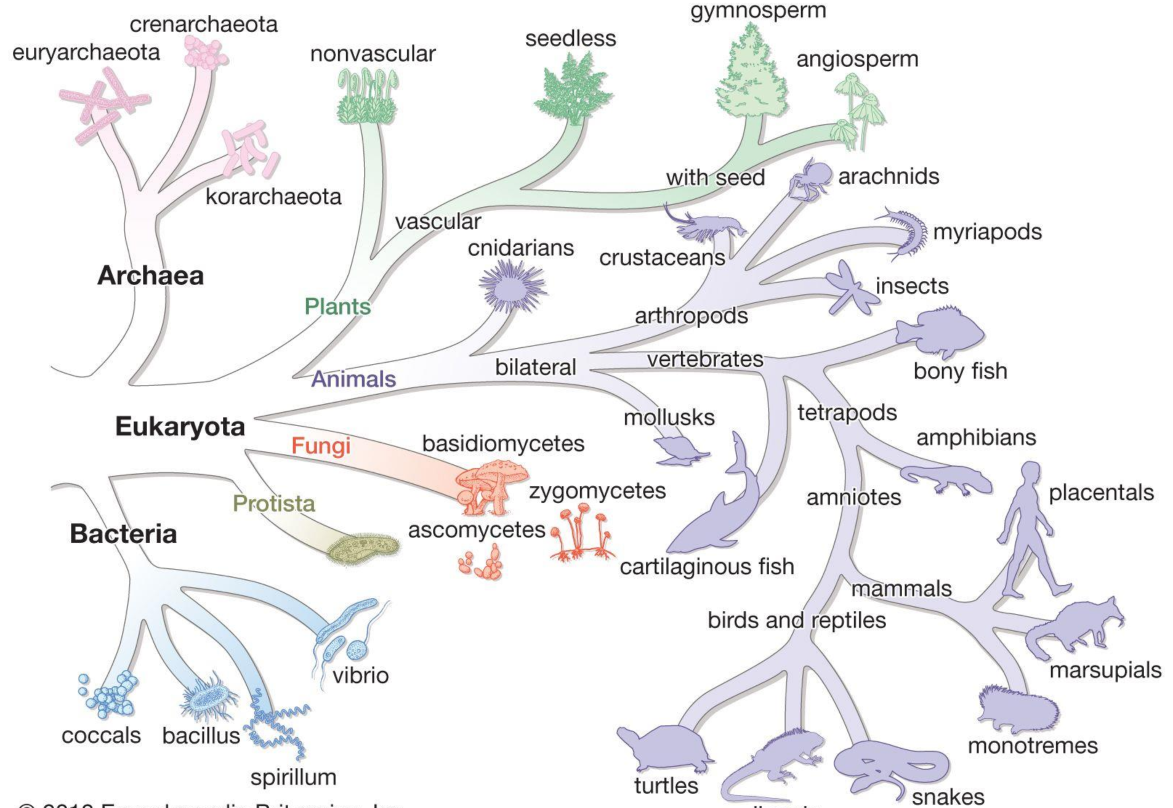
Physical systems





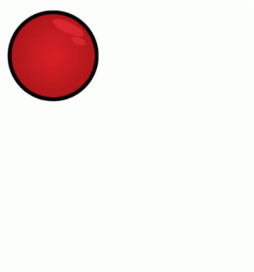
Biological systems

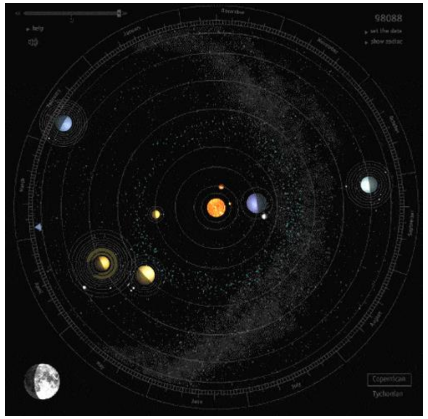




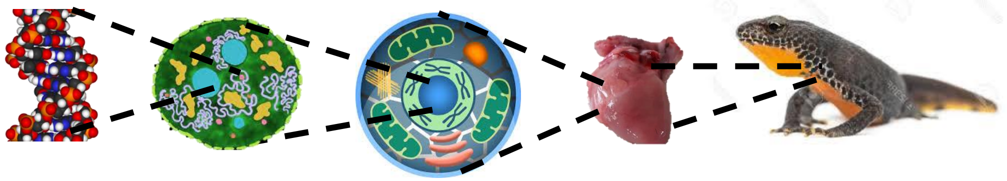
Why is maths everywhere in physics but rare in biology?

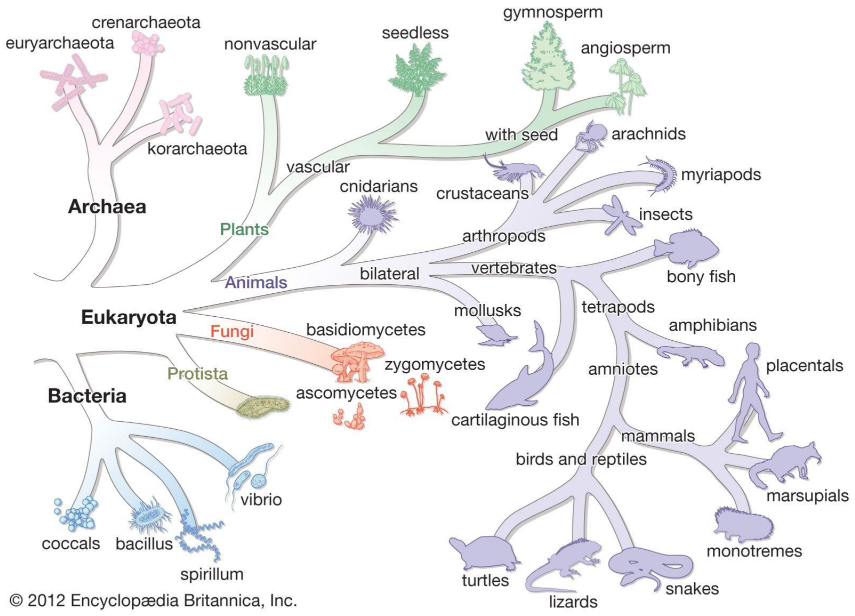
Physical systems





Biological systems





→Biological systems have a **long history** during which they accumulated **information** and **complexity**.

→ It is **difficult to derive general laws** that are true for all biological systems, since they all **depend on their unique history**

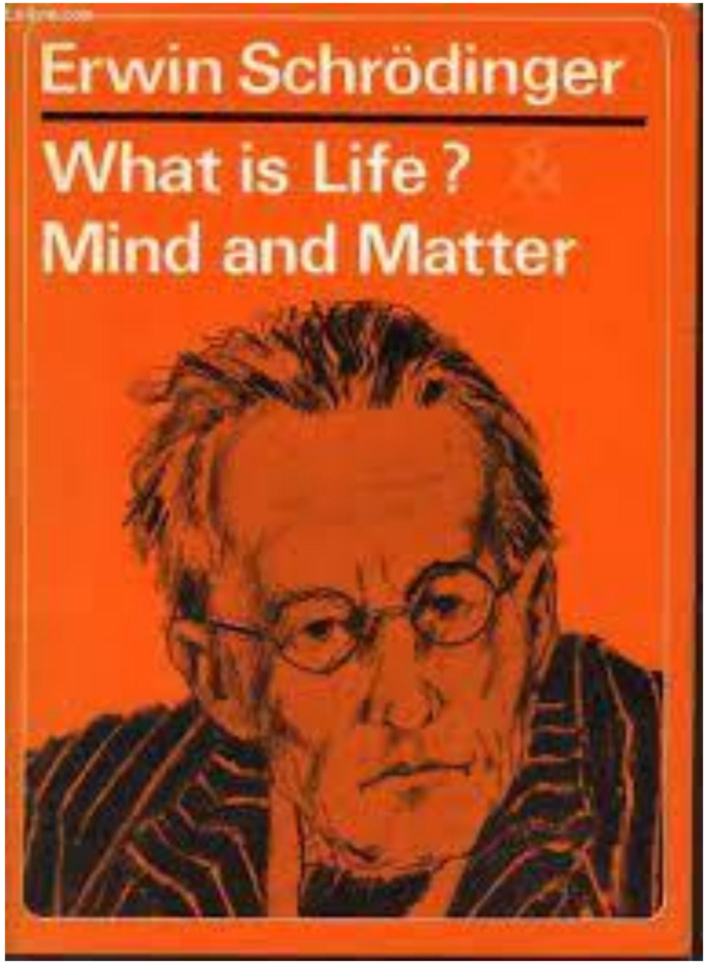
What is biophysics?

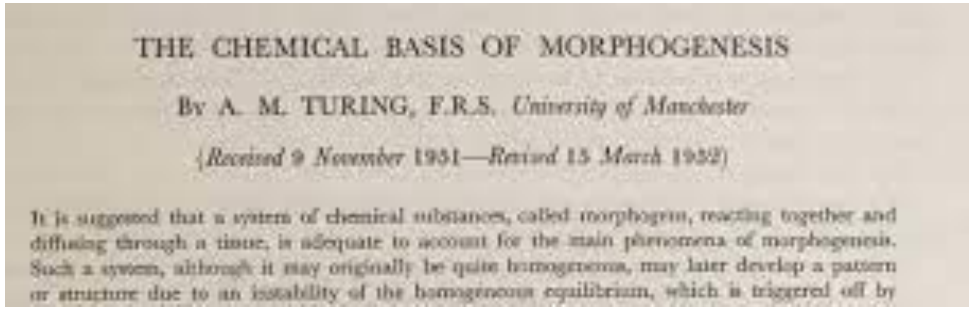
1. The application of tools and traditions typically used in physics to biological phenomena

   → In particular this involves the use of mathematical models

2. The consideration of physical laws when trying to explain biological phenomena

Some historically important examples

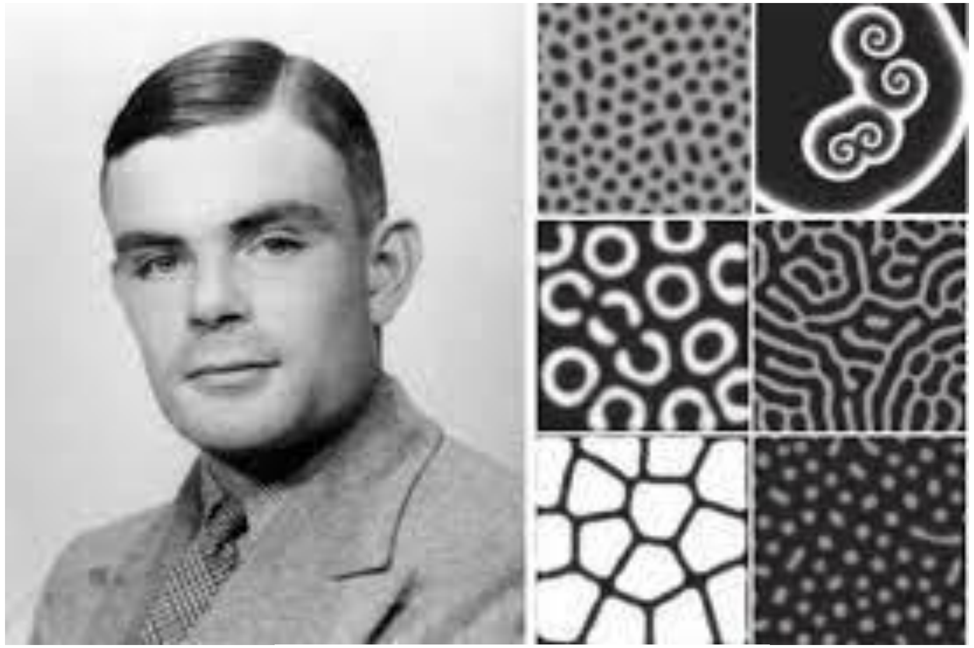


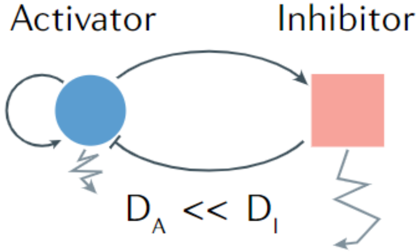

THE CHEMICAL BASIS OF MORPHOGENESIS

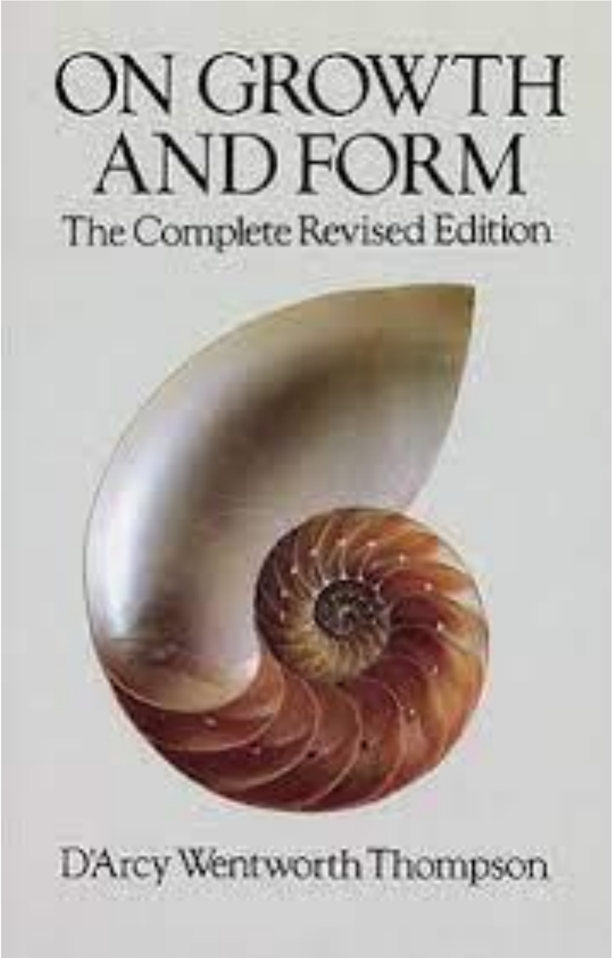
By A. M. TURING, F.R.S. *University of Manchester*

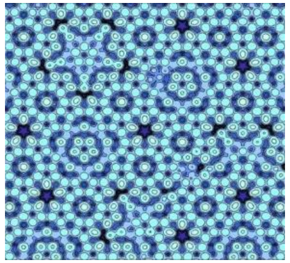
(*Received* 9 *November* 1951—*Revised* 15 *March* 1952)

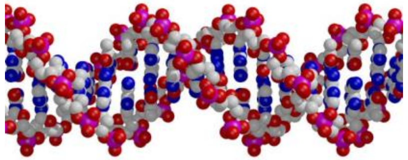
It is suggested that a system of chemical substances, called morphogens, reacting together and diffusing through a tissue, is adequate to account for the main phenomena of morphogenesis. Such a system, although it may originally be quite homogeneous, may later develop a pattern or structure due to an instability of the homogeneous equilibrium, which is triggered off by





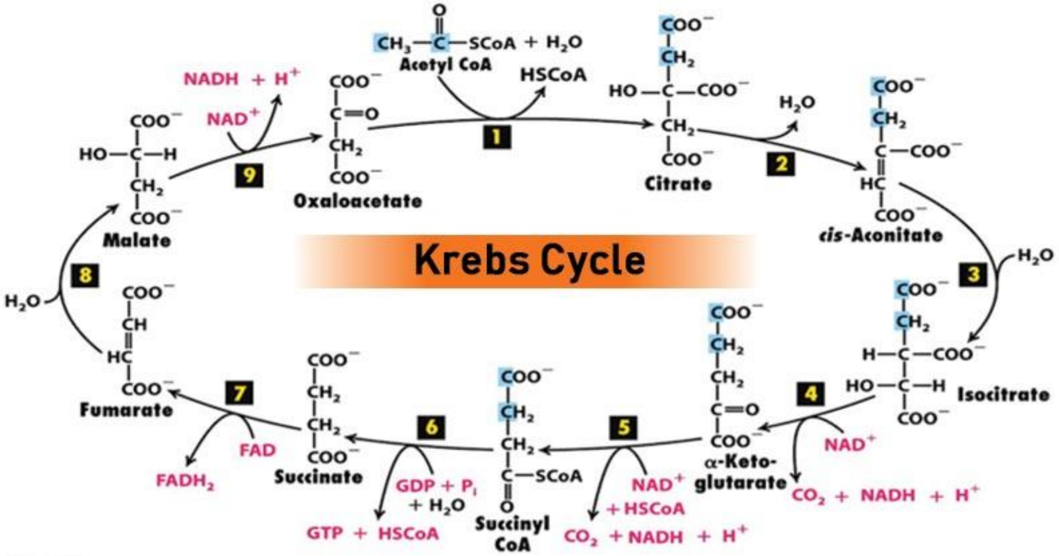






The underappreciated role of mechanics in biology

Life is full of complicated chemistry…

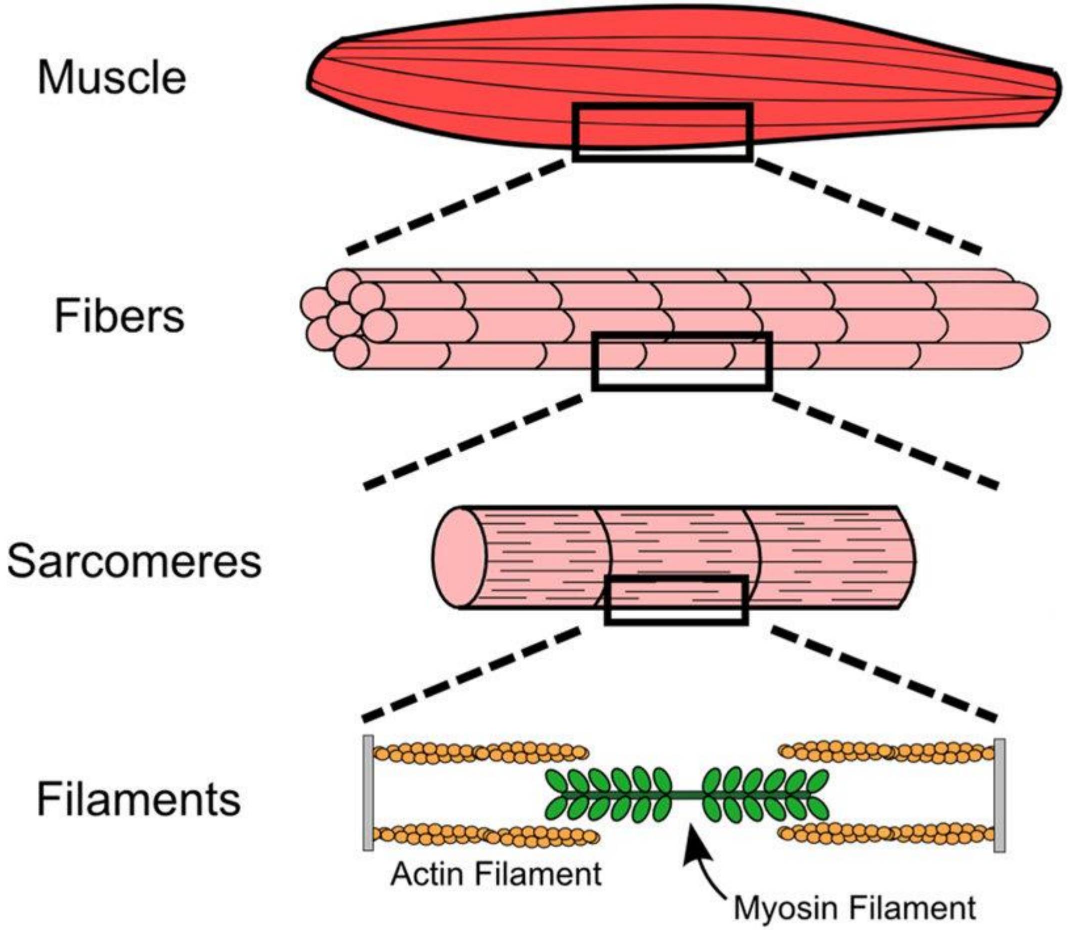


but it needs to interact with the physical world too!

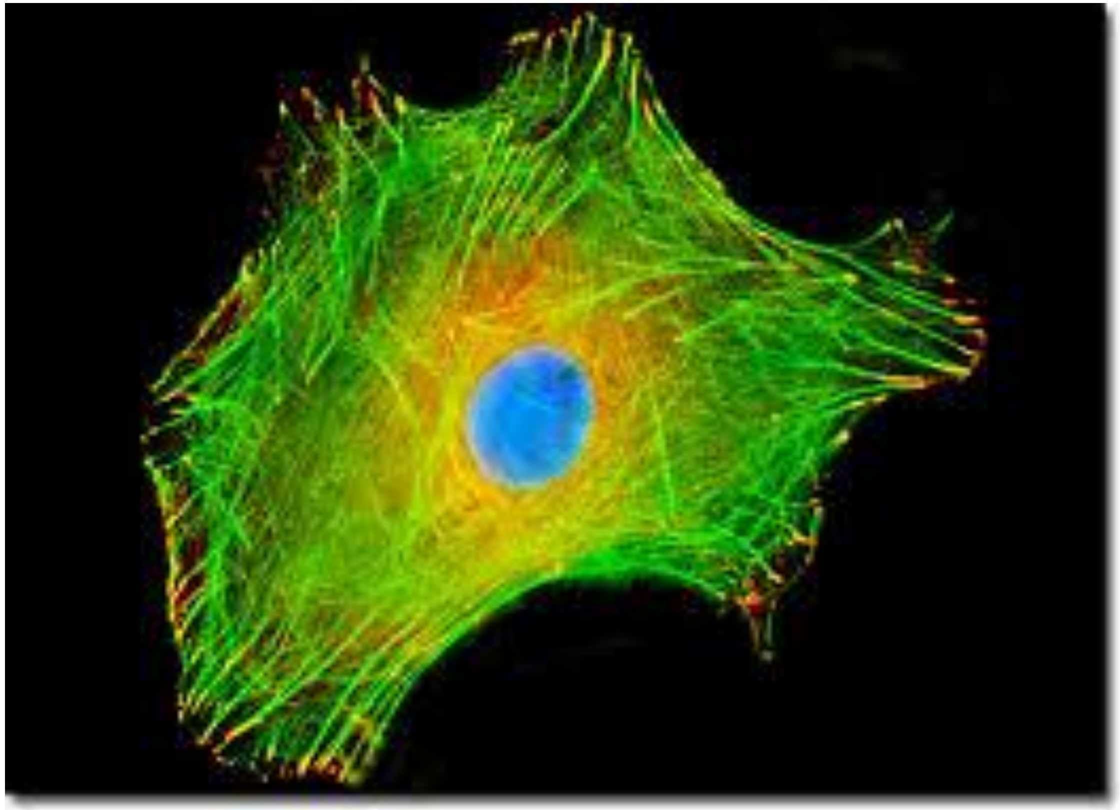
Bridging the gap between chemistry and physics

The actomyosin network

Highly aligned actomyosin filaments allow muscle cells to exert contractile forces



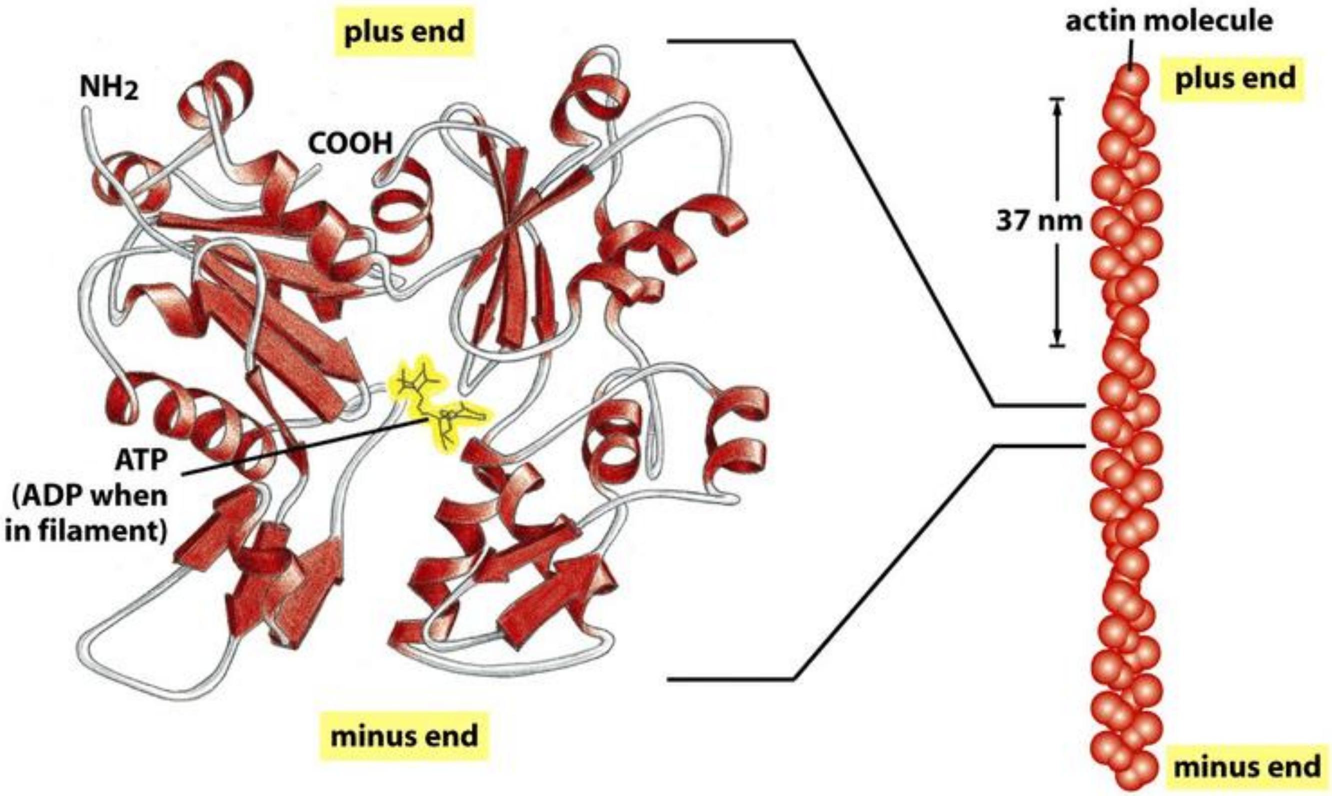
But not only muscles: Essentially all eukaryote cells express actin and myosin and form contractile networks



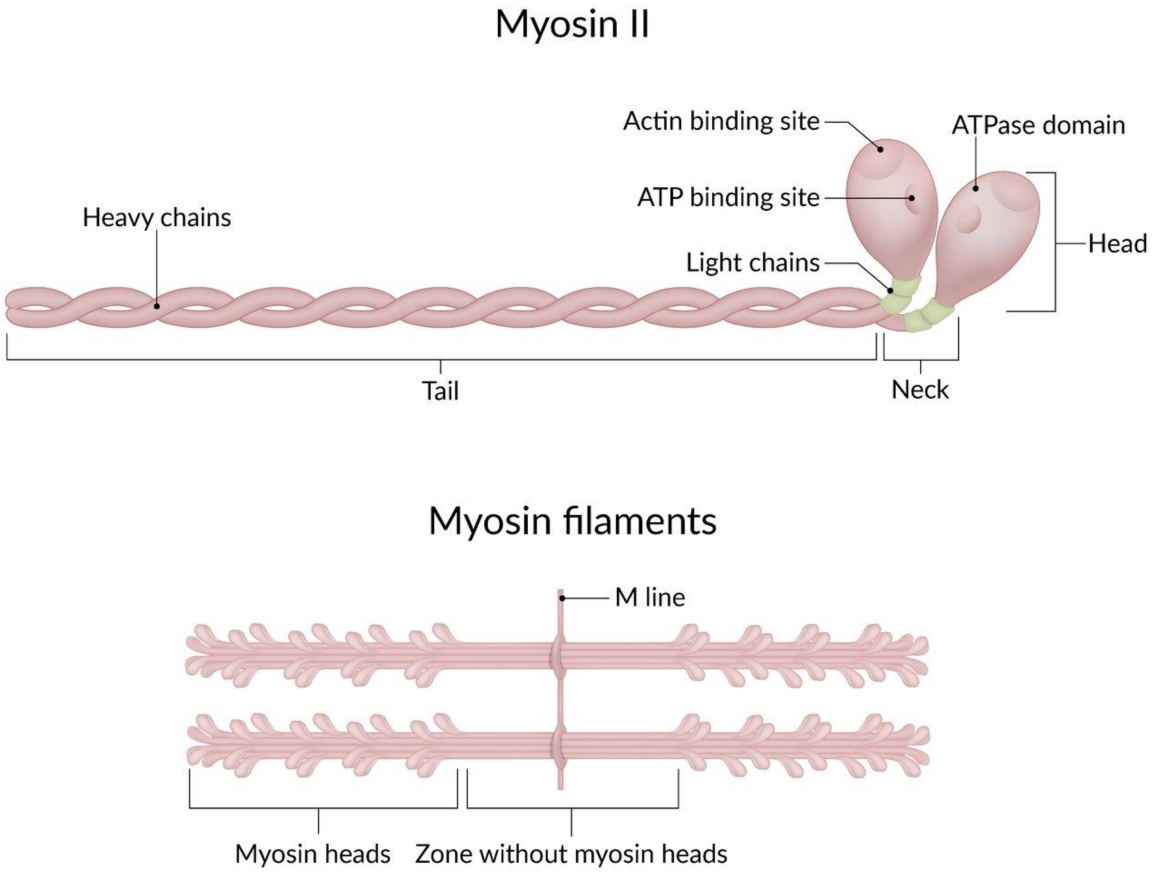
The molecular basis of cell force generation

Force generation of the contractile actomyosin network

Actin monomers polymerize into **polarized** filaments

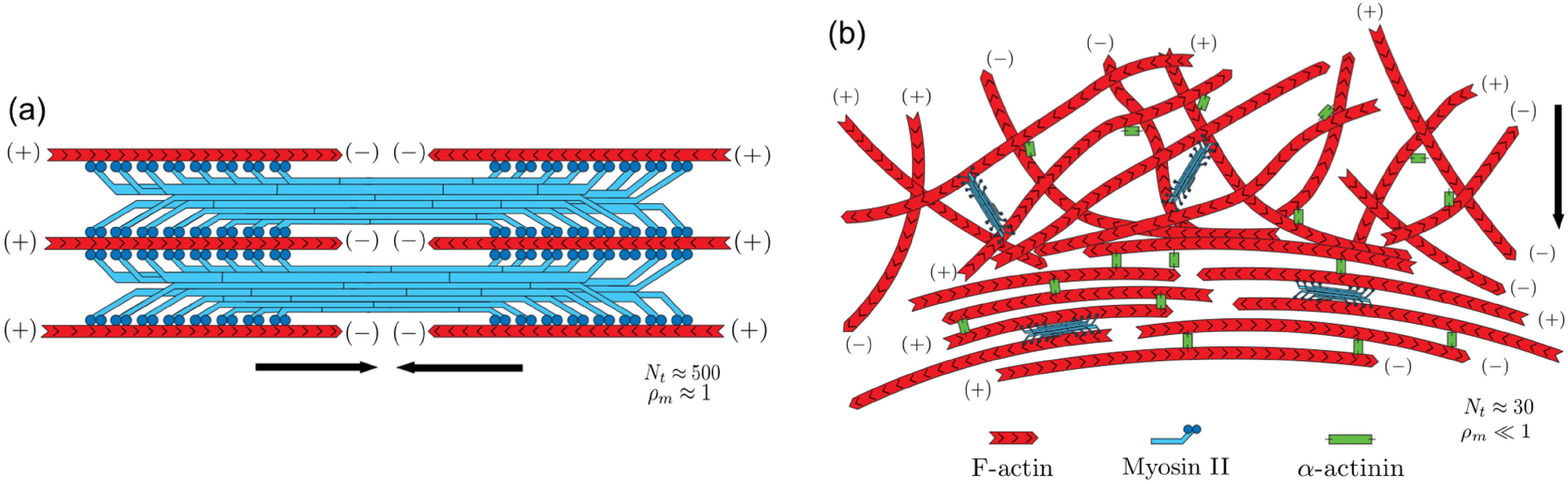


Same goes for myosin



The molecular basis of cell force generation

Force generation of the contractile actomyosin network

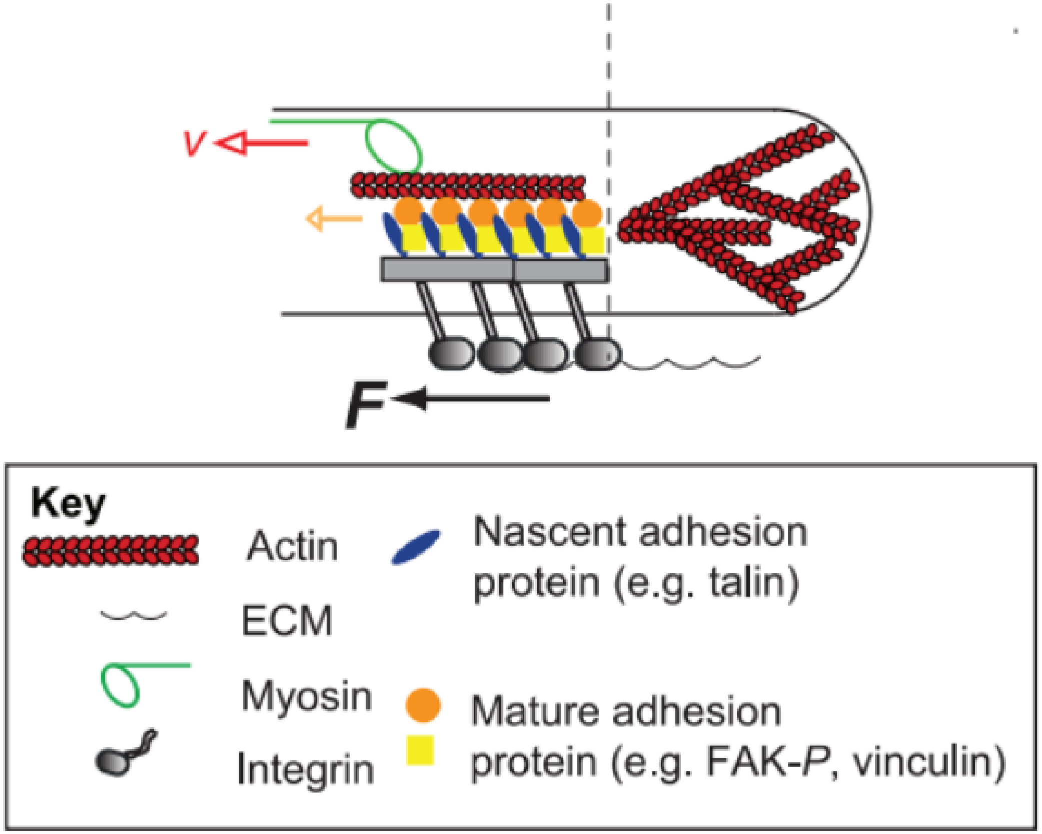


Together, they generate a large variety of contractile networks

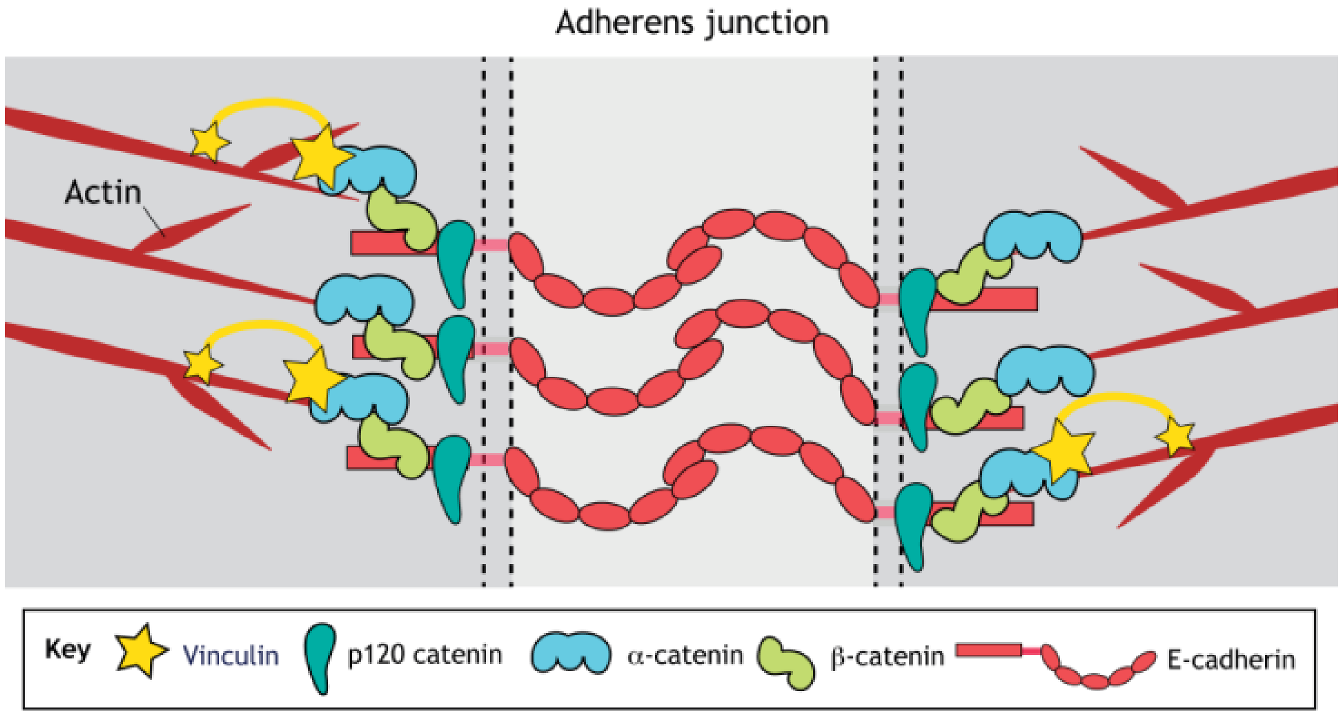
The molecular basis of cell force generation

Transmission of actomyosin forces to the cell surrounding

Cell-matrix adhesion



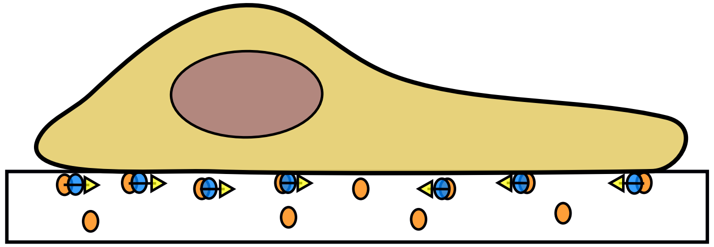
Cell-cell adhesion



The molecular basis of cell force generation

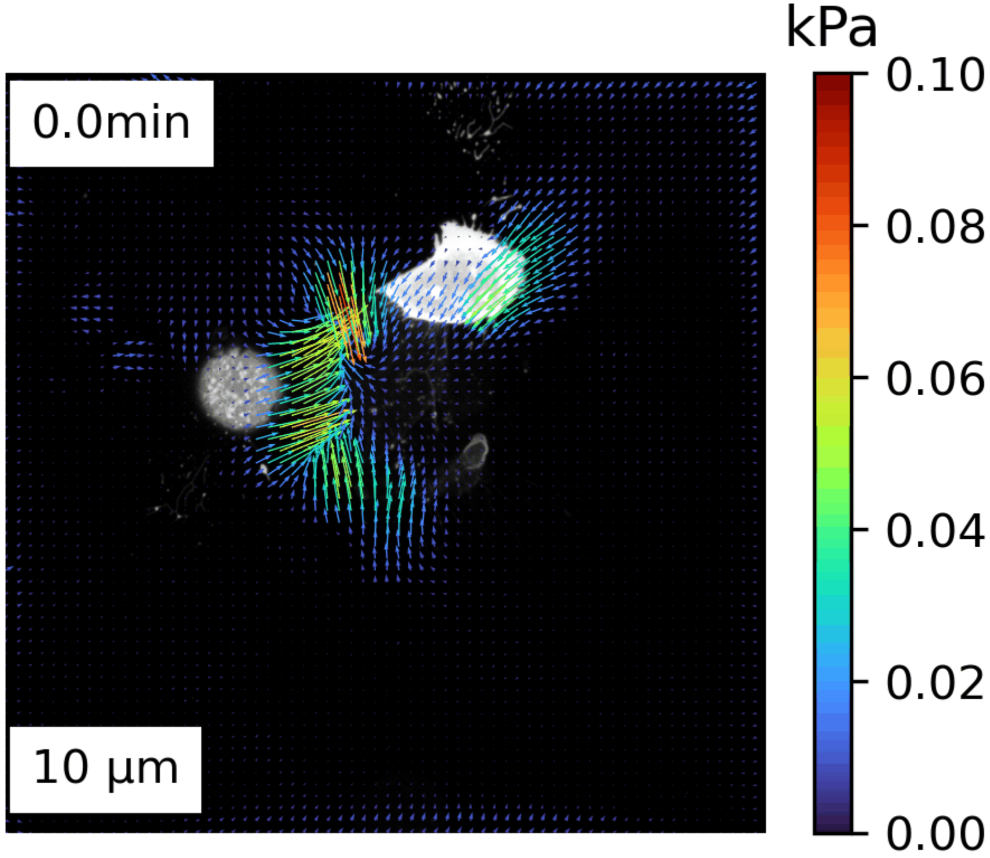
Measuring cell traction forces

Put cells on soft substrate containing fluorescent markers



Measure marker displacements

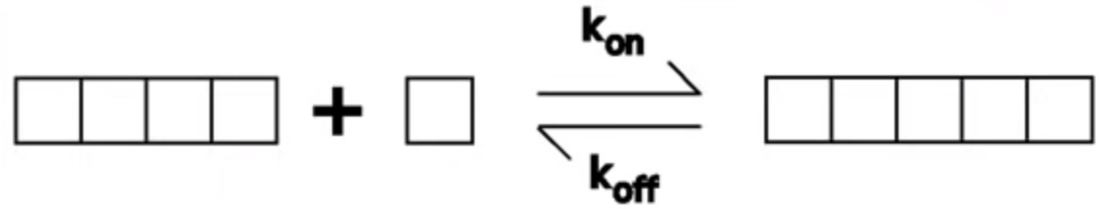
Calculate traction forces



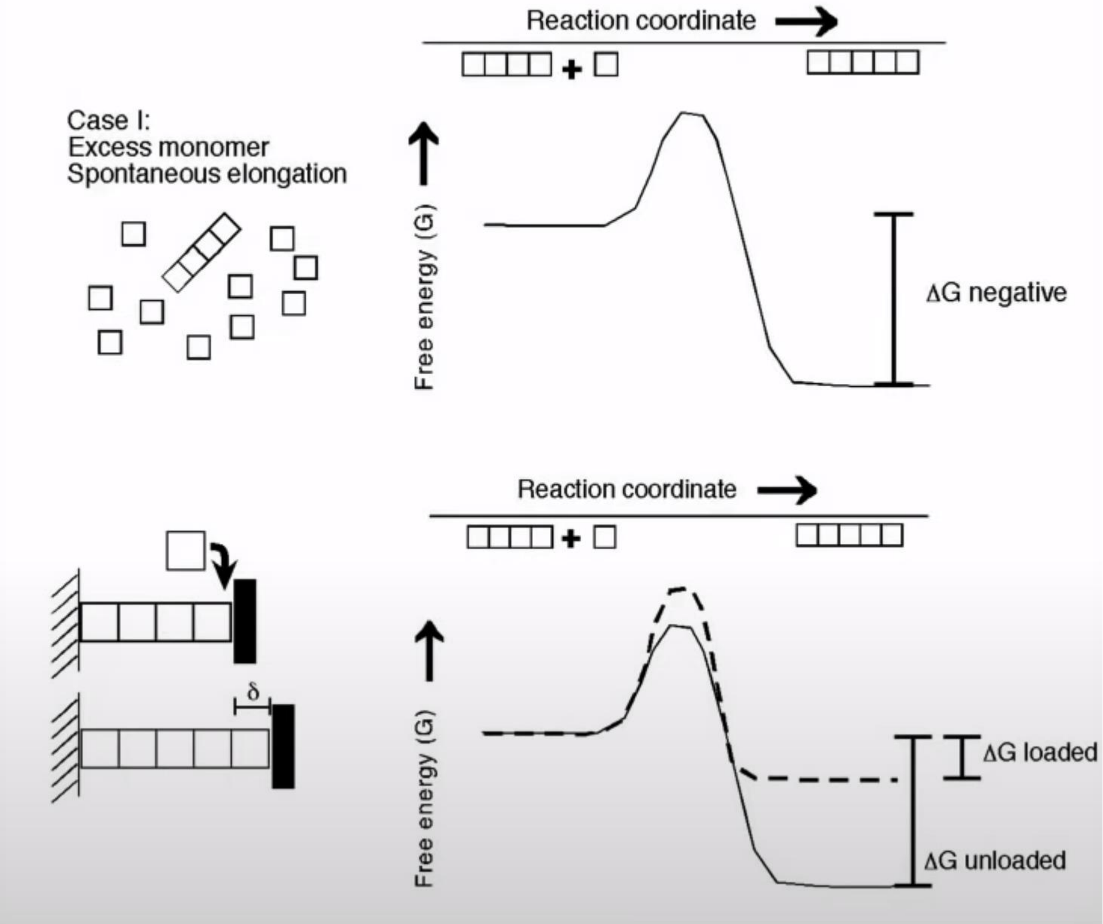
The molecular basis of cell force generation

Force generation by actin polymerization

Actin polymerization is always favored, since monomers are always in excess



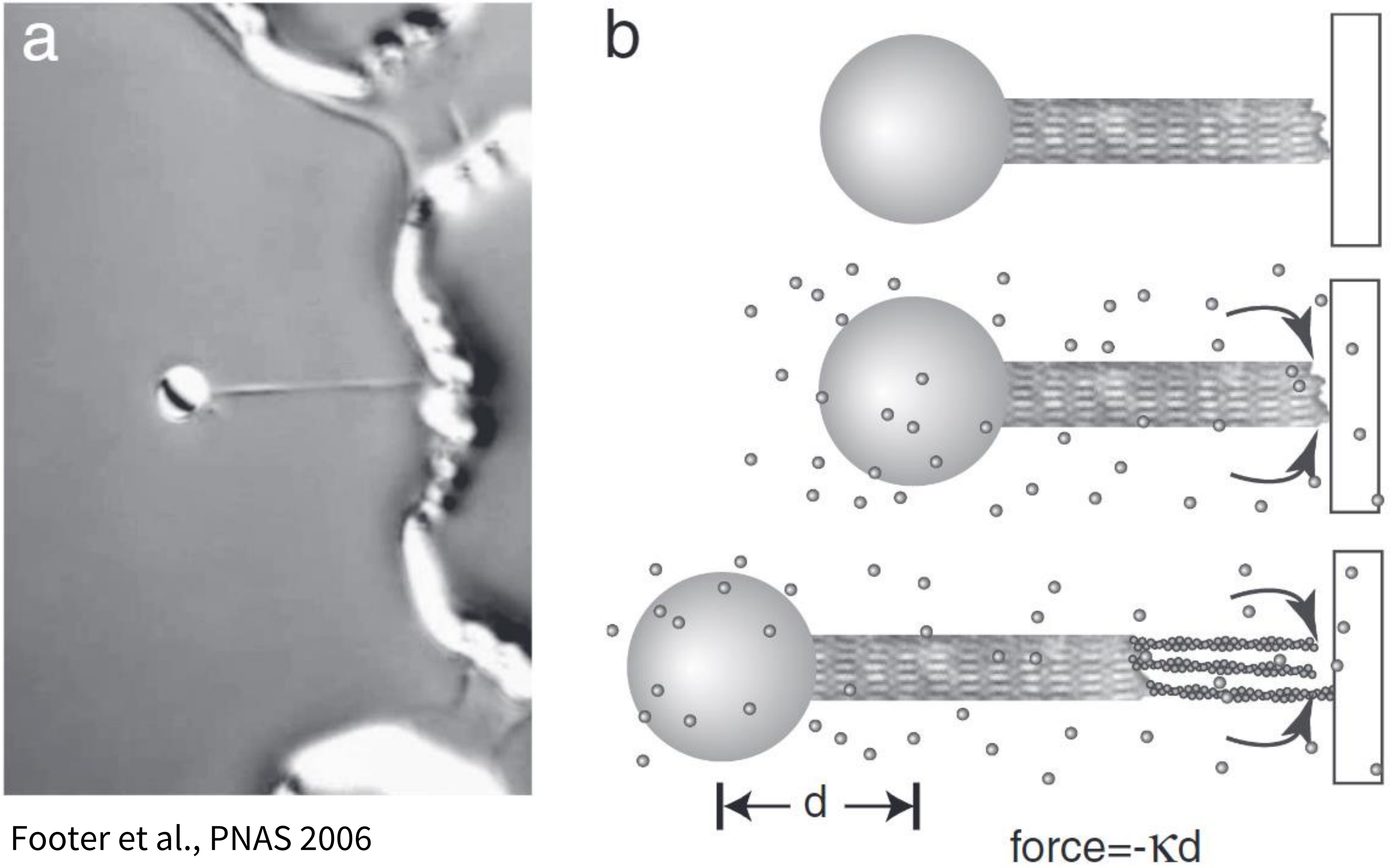
Which means that actin filaments can polymerize against an external load



Adapted from Hill & Kirschner, 1982, *Int. Rev. Cytol.* 78: 1-125

The molecular basis of cell force generation

Measuring force generation by actin polymerization



Footer et al., PNAS 2006

→This actin polymerization force can be measured with an optical trap

→The force was found to be about 1pN per actin filament

So cells pull and push on their surroundings… but why?

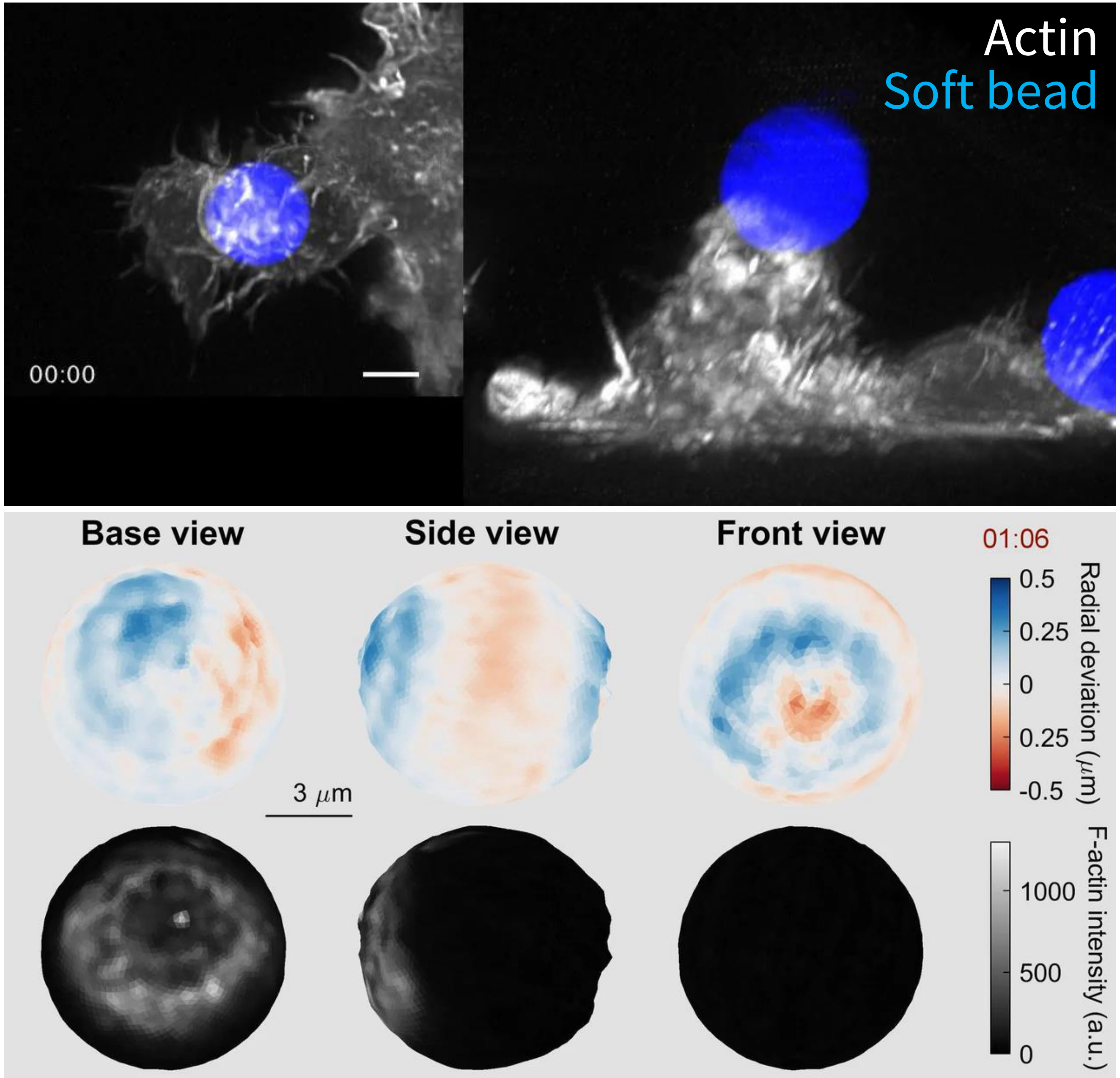
… to move around (i.e. to migrate)

YouTube,
The Science
Tutorials Channel

So cells pull and push on their surroundings... but why?

… to eat

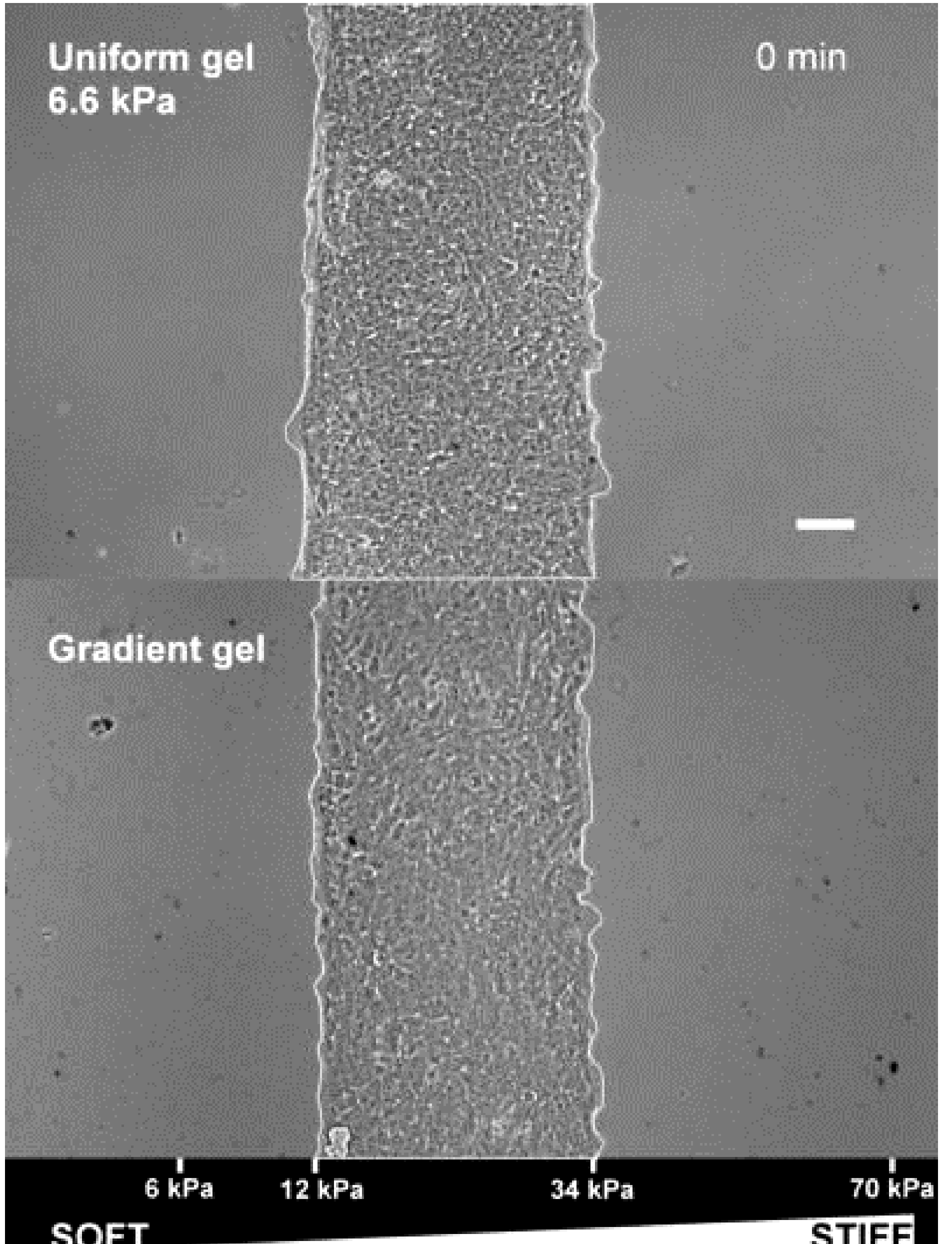
(i.e. to do phagocytosis)



Vorselen et al., eLife 2021

So cells pull and push on their surroundings… but why?

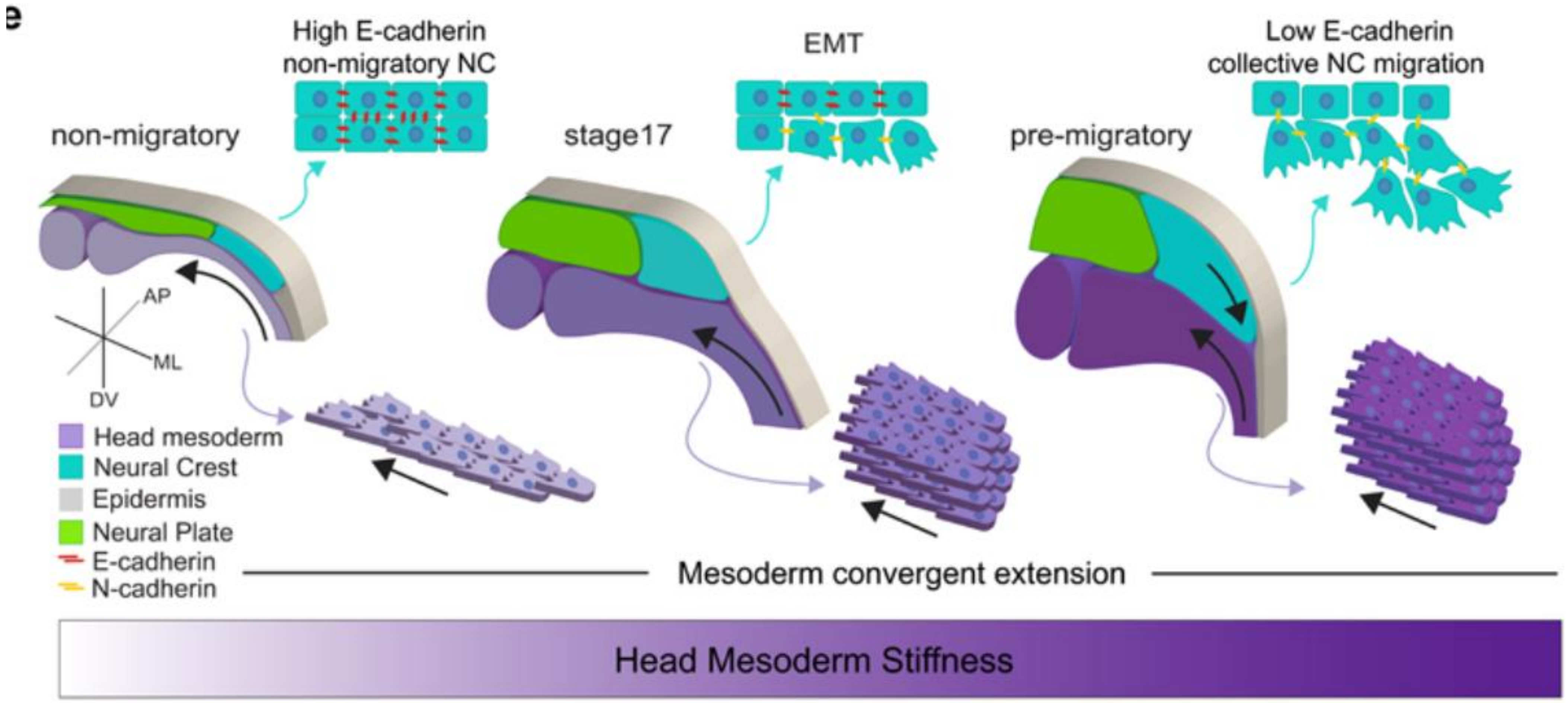
… to feel their environment
(i.e. to probe it’s mechanical properties)



Sunyer et al., Science 2016

So cells pull and push on their surroundings… but why?

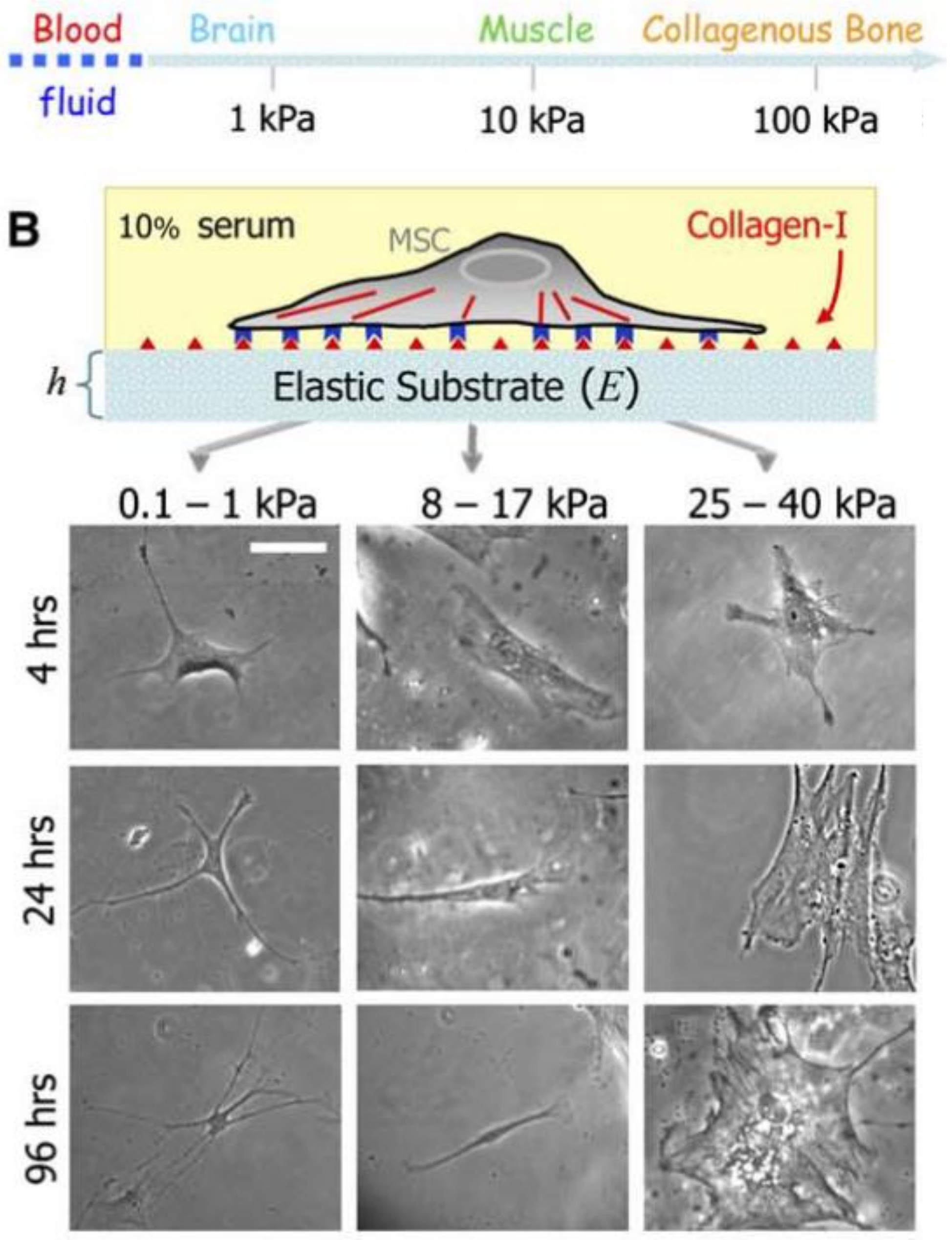
Substrate stiffness is also an important guidance cue during morphogenesis



Barriga et al., Nature 2018

So cells pull and push on their surroundings... but why?

Substrate stiffness can even induce cell differentiation!

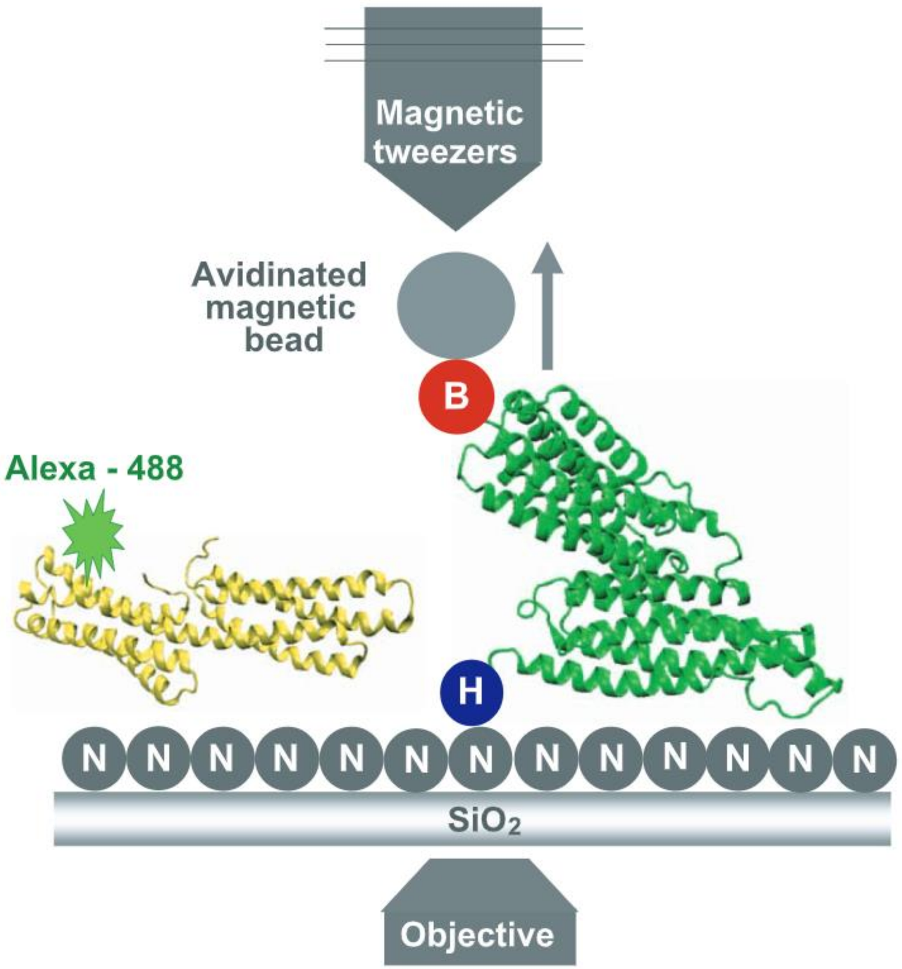


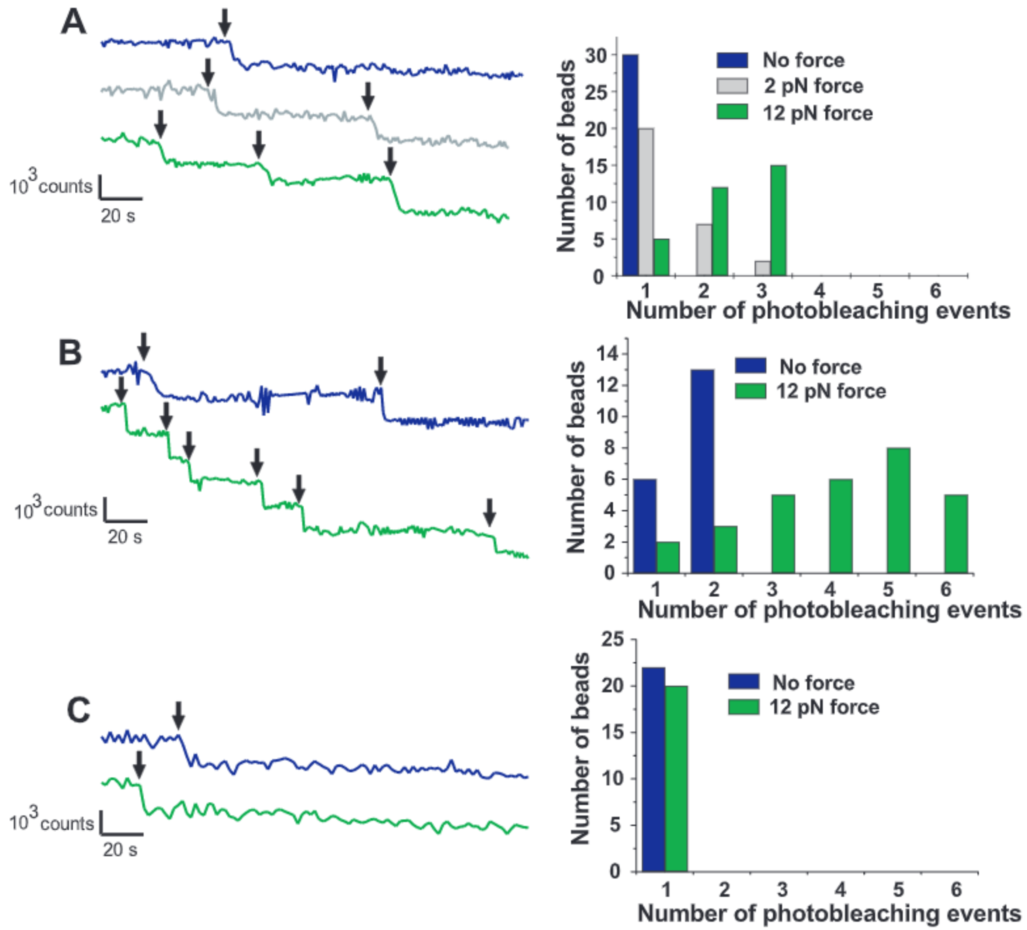
Engler et al., Cell 2006

How can a mechanical stimulus, such as substrate rigidity, influence cell behavior?

→ 1st necessary ingredient: A mechanosensor

Mechanotransduction: Going back from physics to chemistry



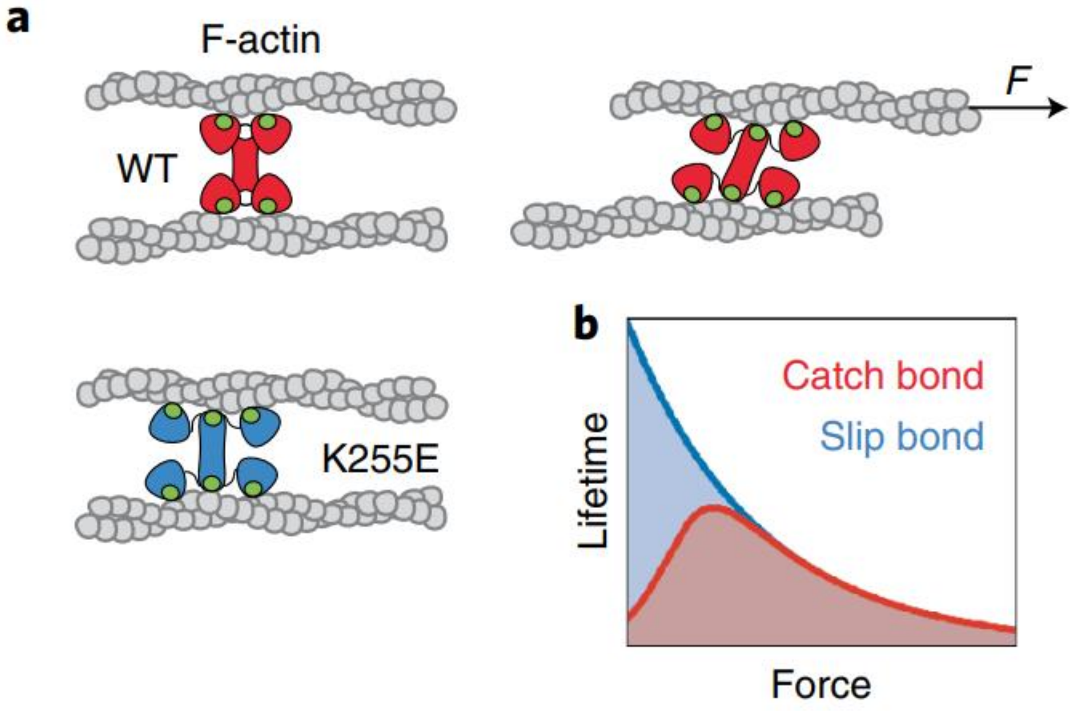


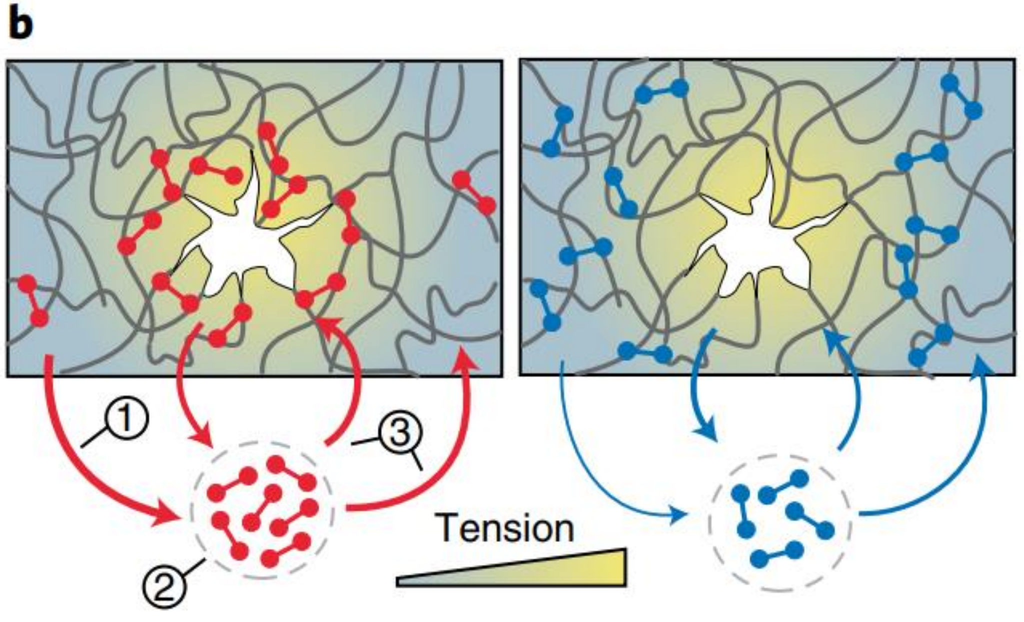
Rio et al., Science 2009

→ Mechanical stretching of talin unveils cryptic binding sites for Vinculin

Mechanotransduction: Going back from physics to chemistry

Many other cytoskeletal proteins have similar catch-bond mechanisms



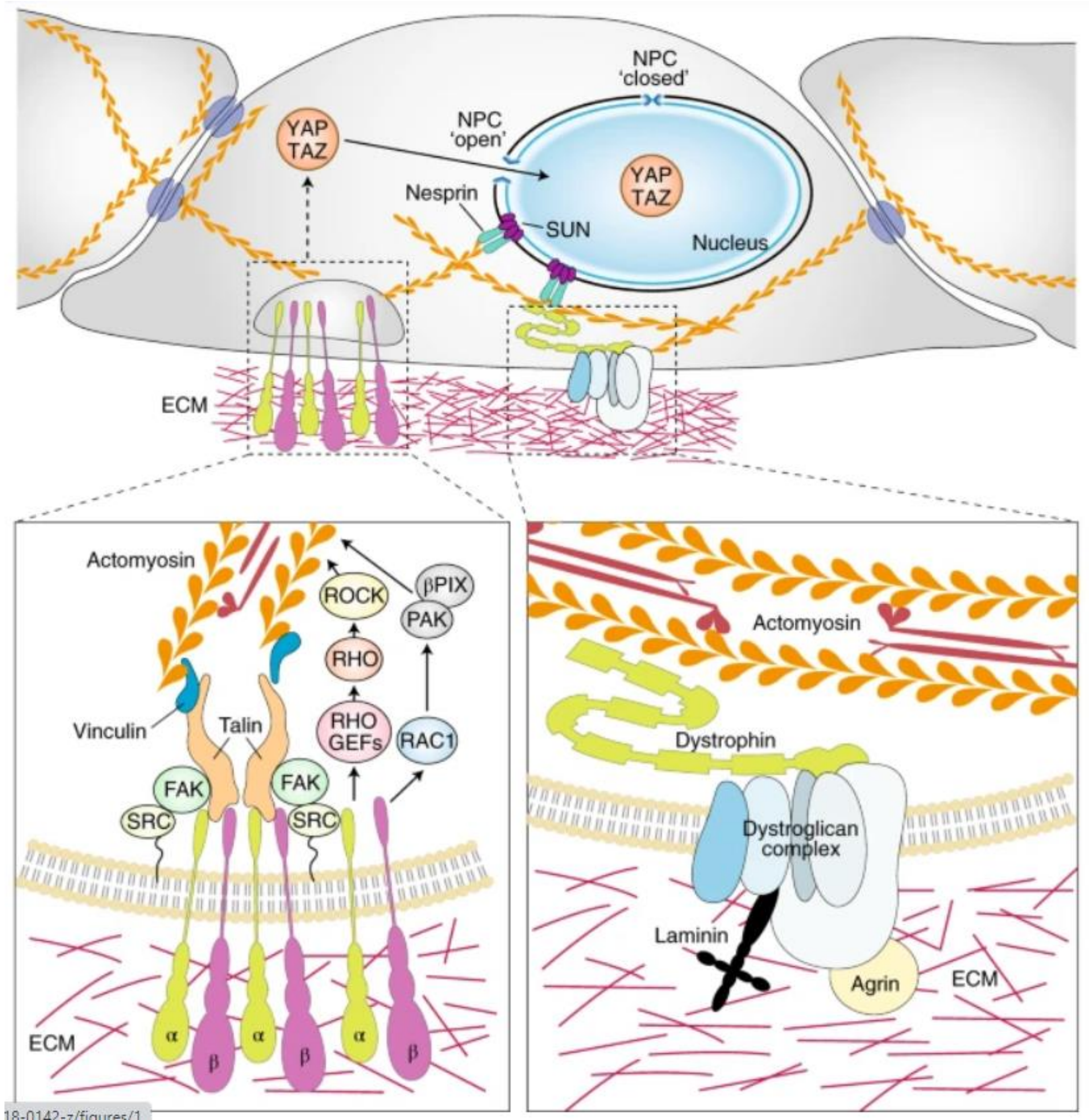


Mulla et al., Nature Materials 2022

→ This allows the cytoskeleton to adapt to external tensions

OK, but how does that explain mechanically induced differentiation?

Mechanotransduction: Going back from physics to chemistry

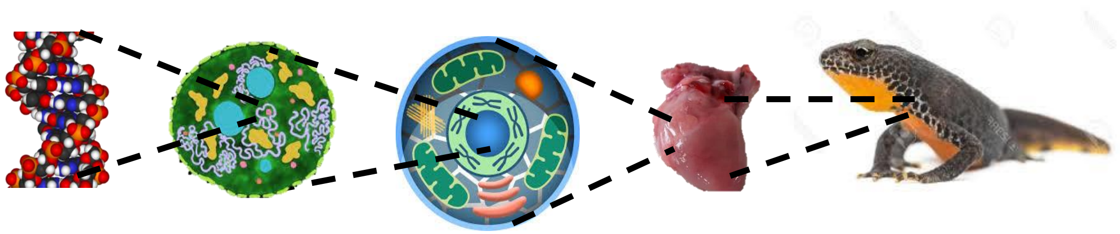


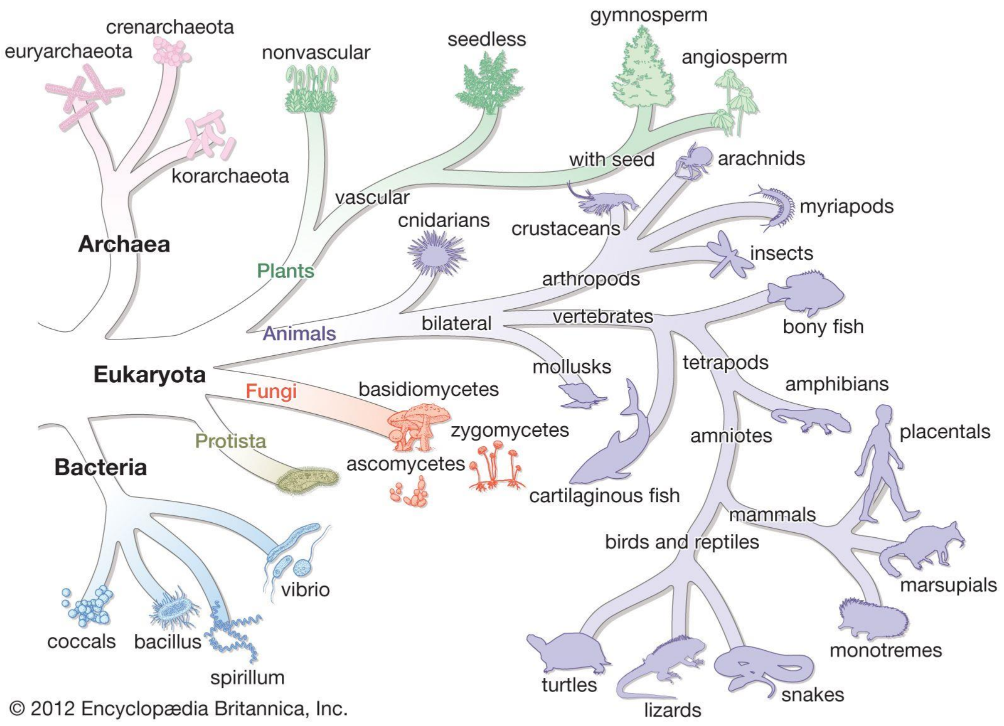
→YAP/TAZ are major transcription factors which enter the nucleus through signaling pathways from focal and cell-cell adhesions

→These pathways are numerous and complex and still subject of intensive research

Totaro et al. Nature Cell Biology 2018

What does all this mean for the bigger picture?



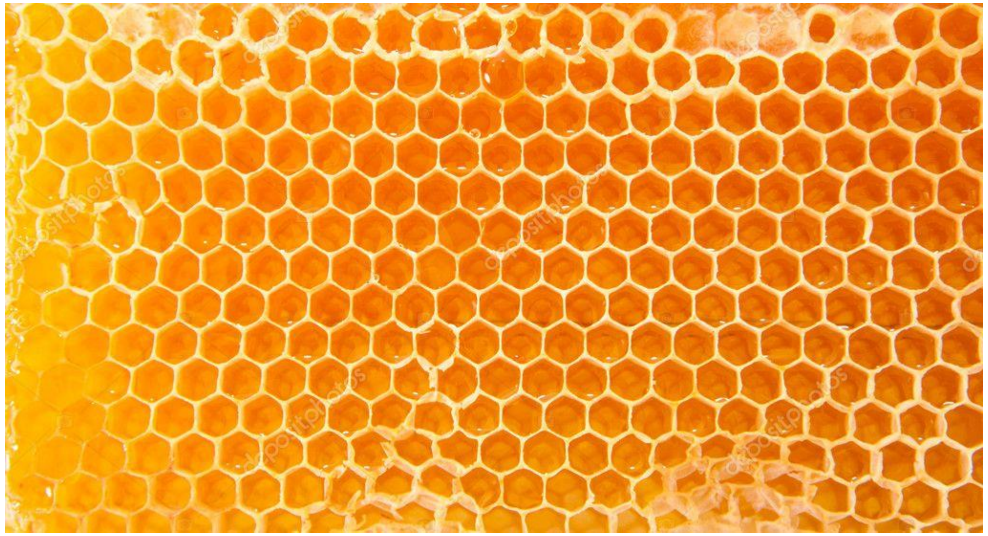


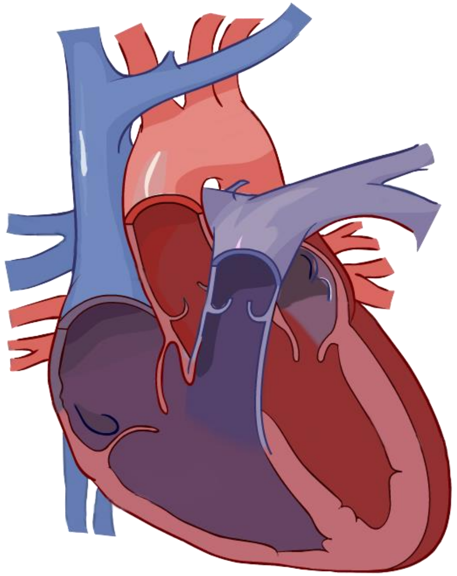
→Biological systems have a **long history** during which they accumulated **information** and **complexity**.

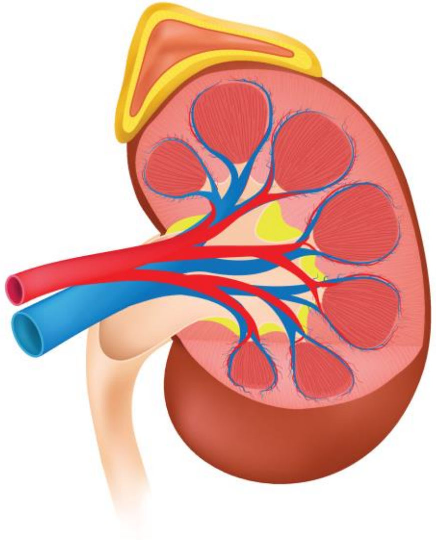
→These complex systems emerge from an interplay of **highly connected chemical** but also **mechanical signaling network**

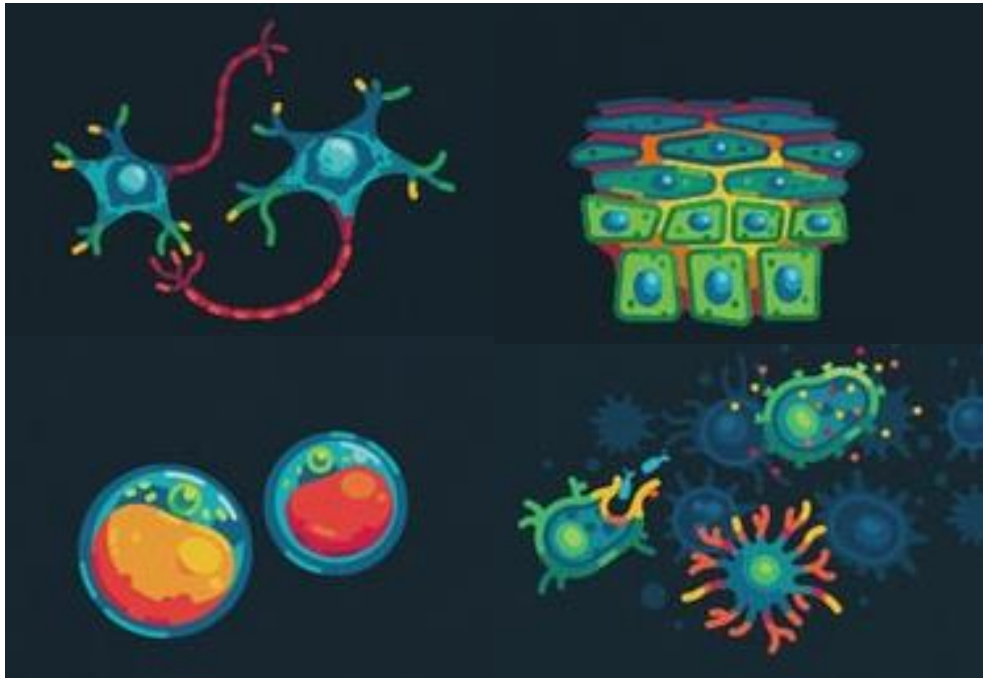
→These different types of signals can be **transduced** into each other through **molecular motors** and **mechanosensor molecules**

A third player in the ring: Geometry



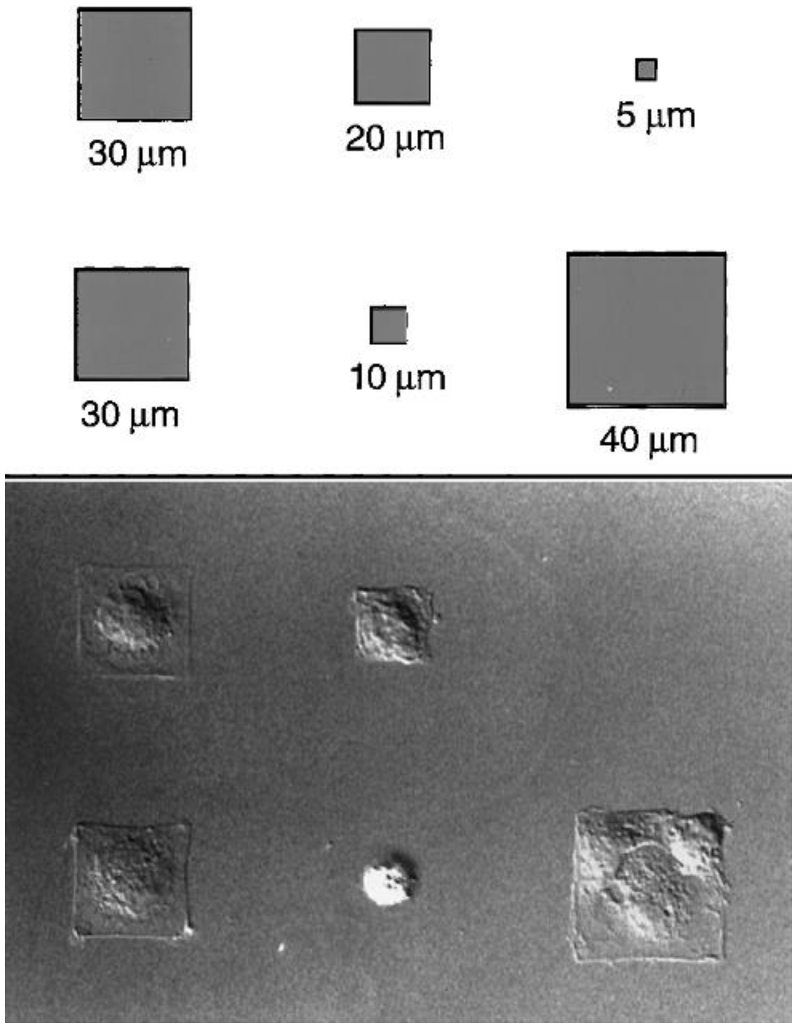


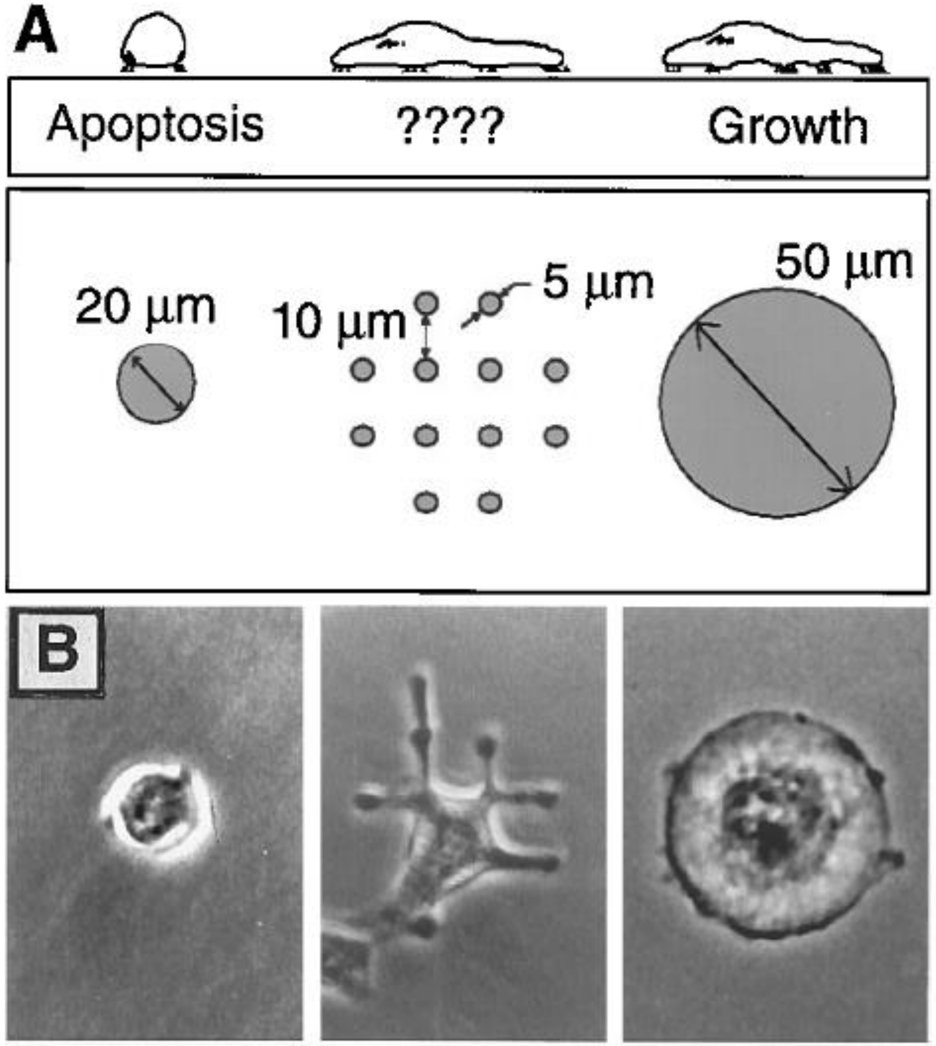




A third player in the ring: Geometry

Geometric control of cell life and death

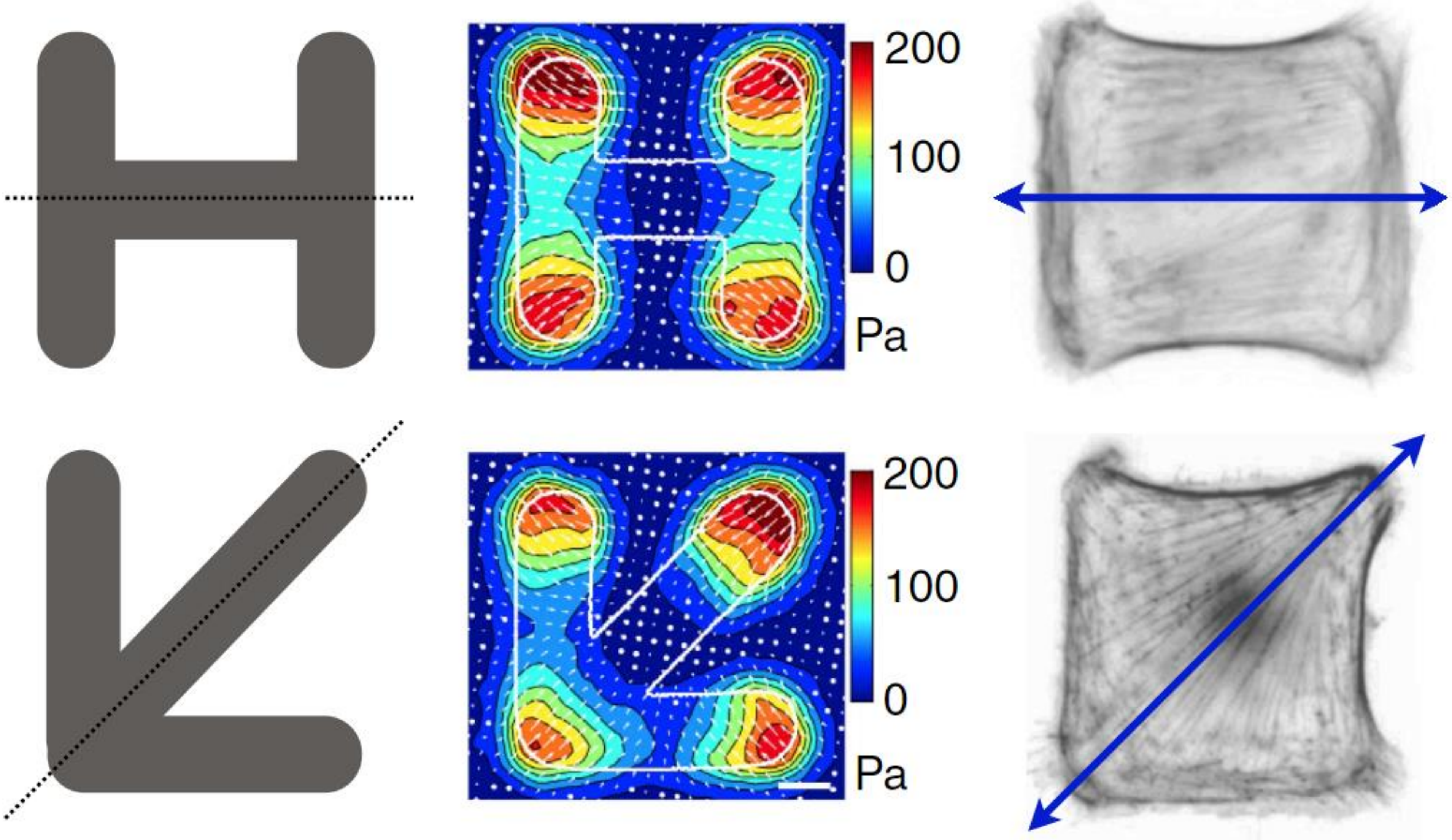




Chen et al., Science 1997

→ Cell shape influences apoptosis!

A third player in the ring: Geometry

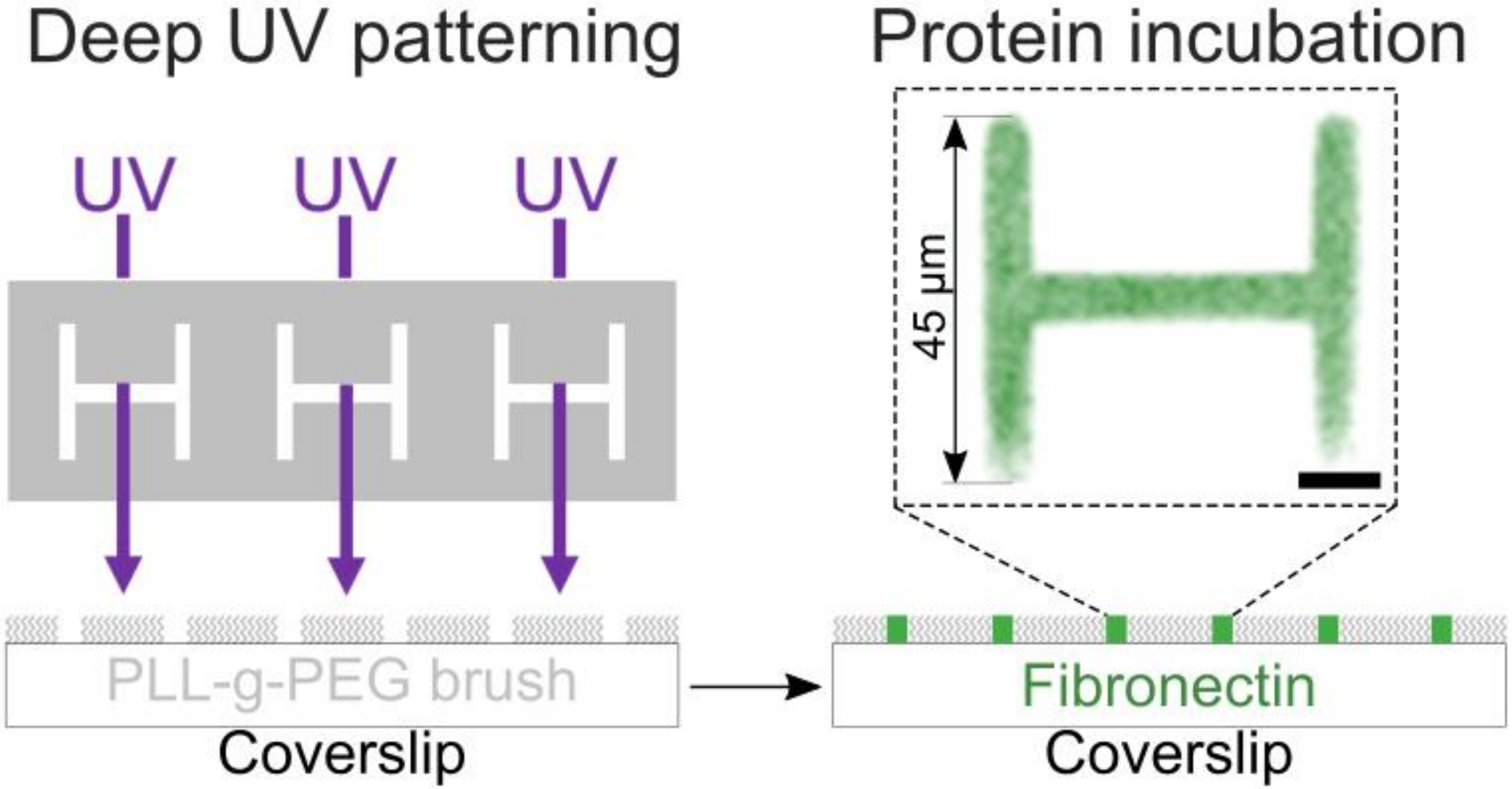


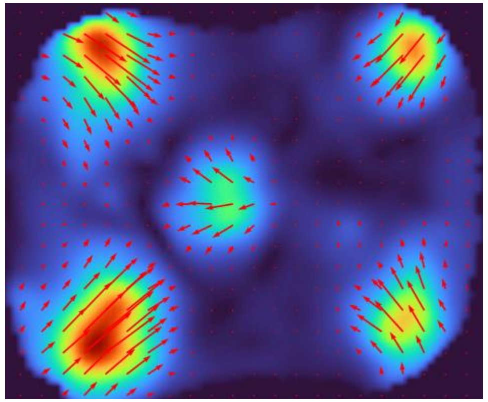
Mandal et al. Nature comm. 2014

→ Substrate geometry influences force and actin orientation

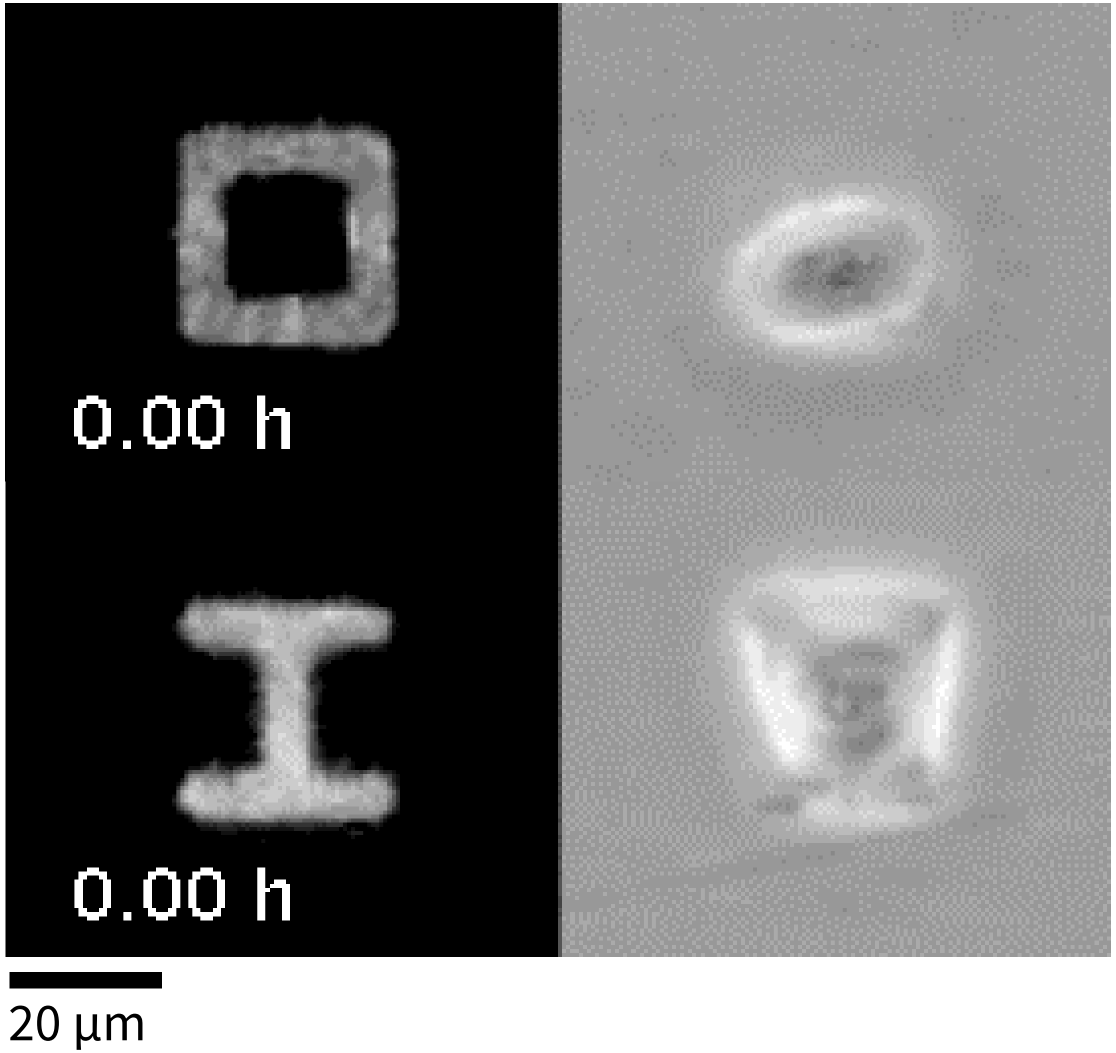
A third player in the ring: Geometry

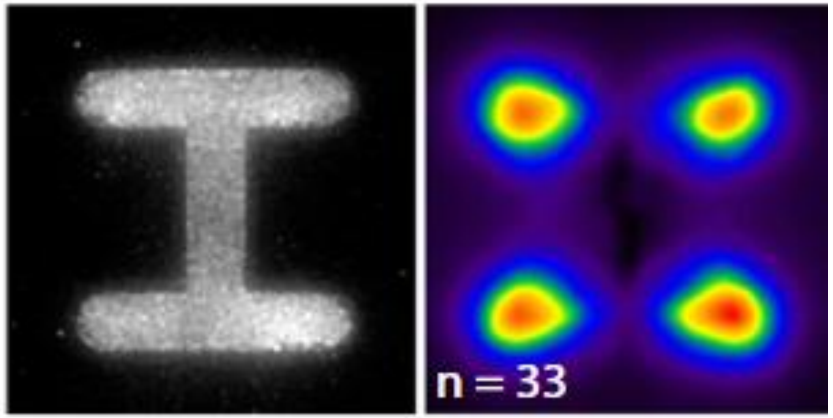
Micropatterns to study interplay between geometry and mechanics





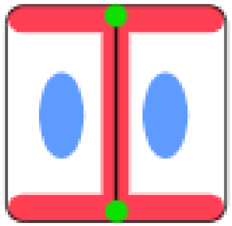
A third player in the ring: Geometry



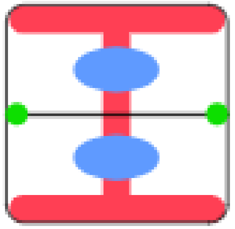


inter-cellular junctions **in contact with** the ECM

inter-cellular junctions **away from** the ECM



high inter-cellular forces
high intra-cellular forces
moving inter-cellular junction

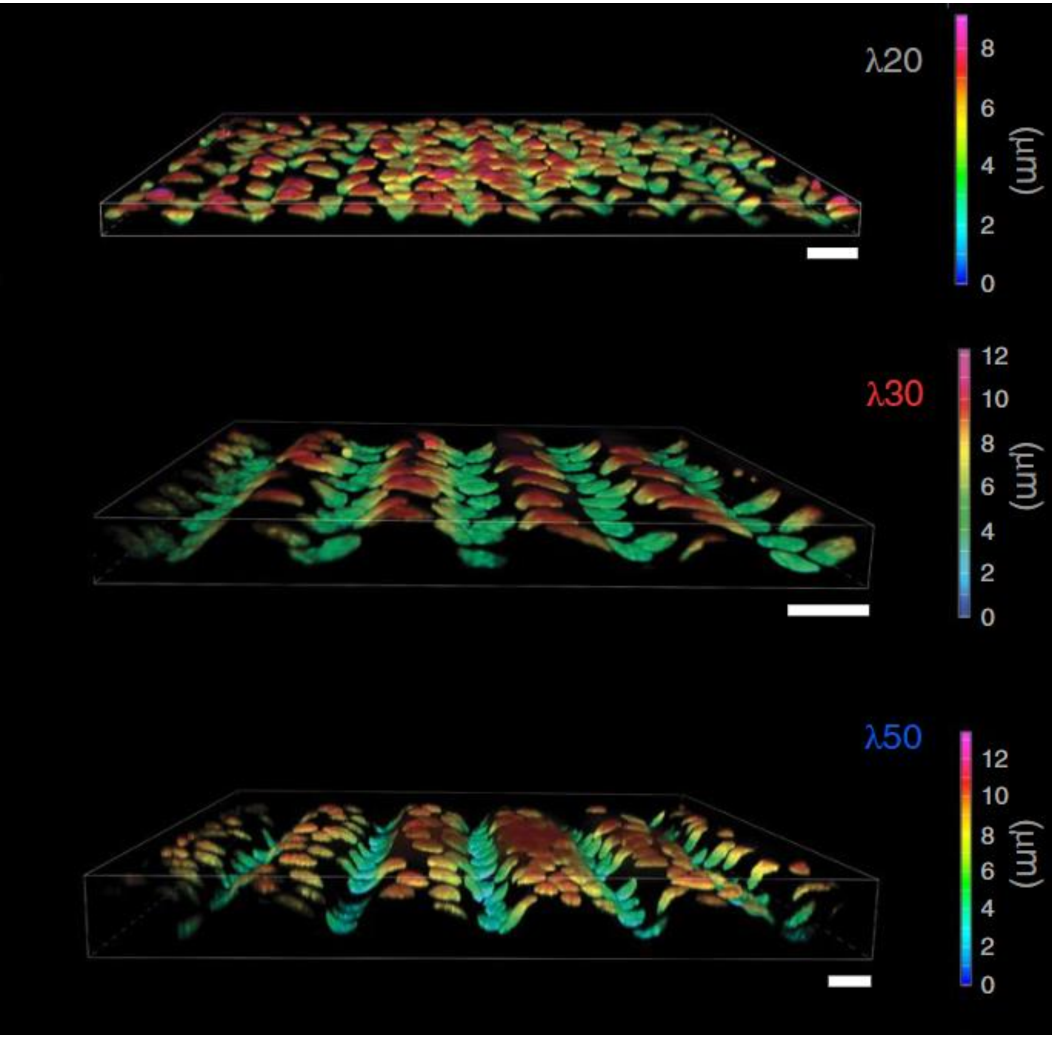


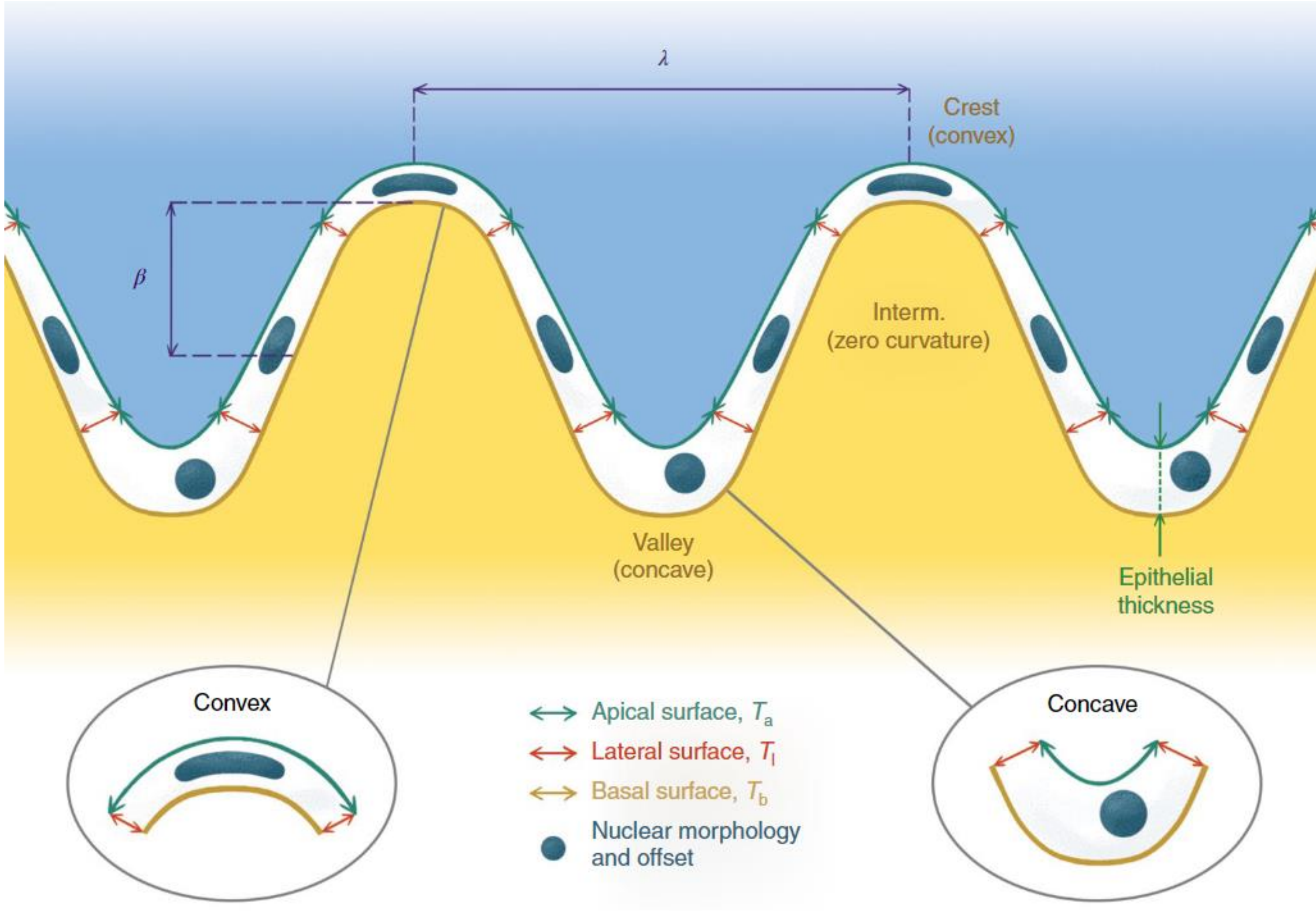
low inter-cellular forces
low intra-cellular forces
non moving inter-cellular junction

Tseng et al., PNAS 2012

→Shape influences cell tension, which influences multicellular architecture

A third player in the ring: Geometry

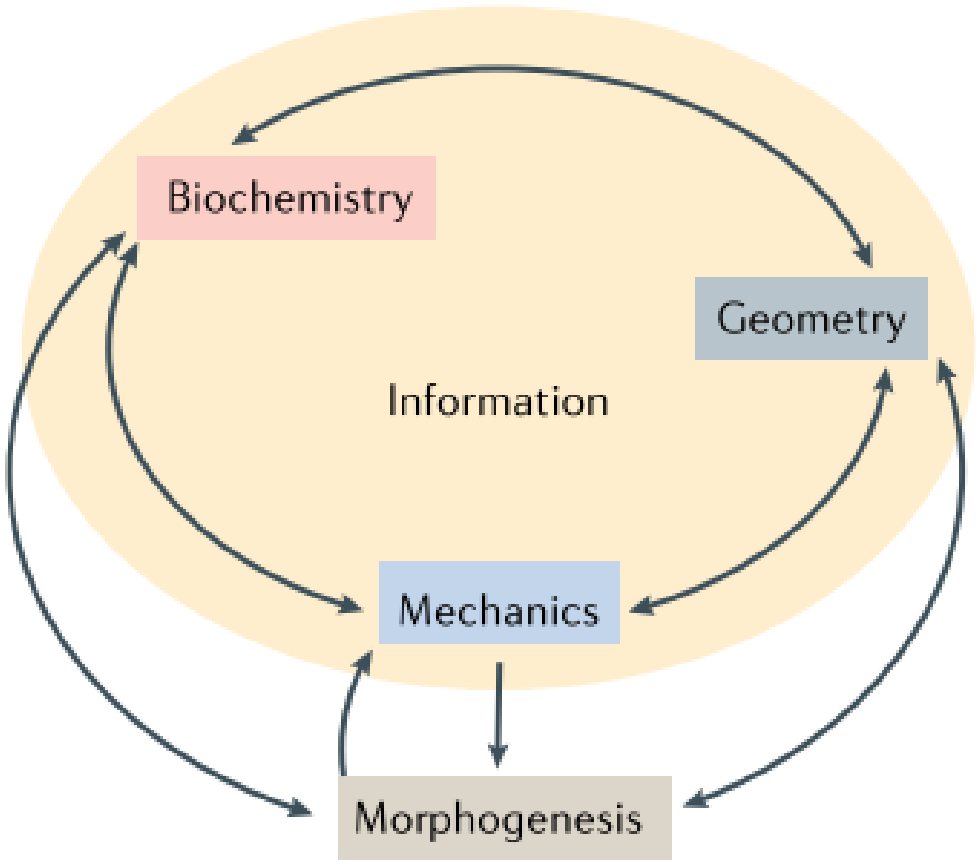




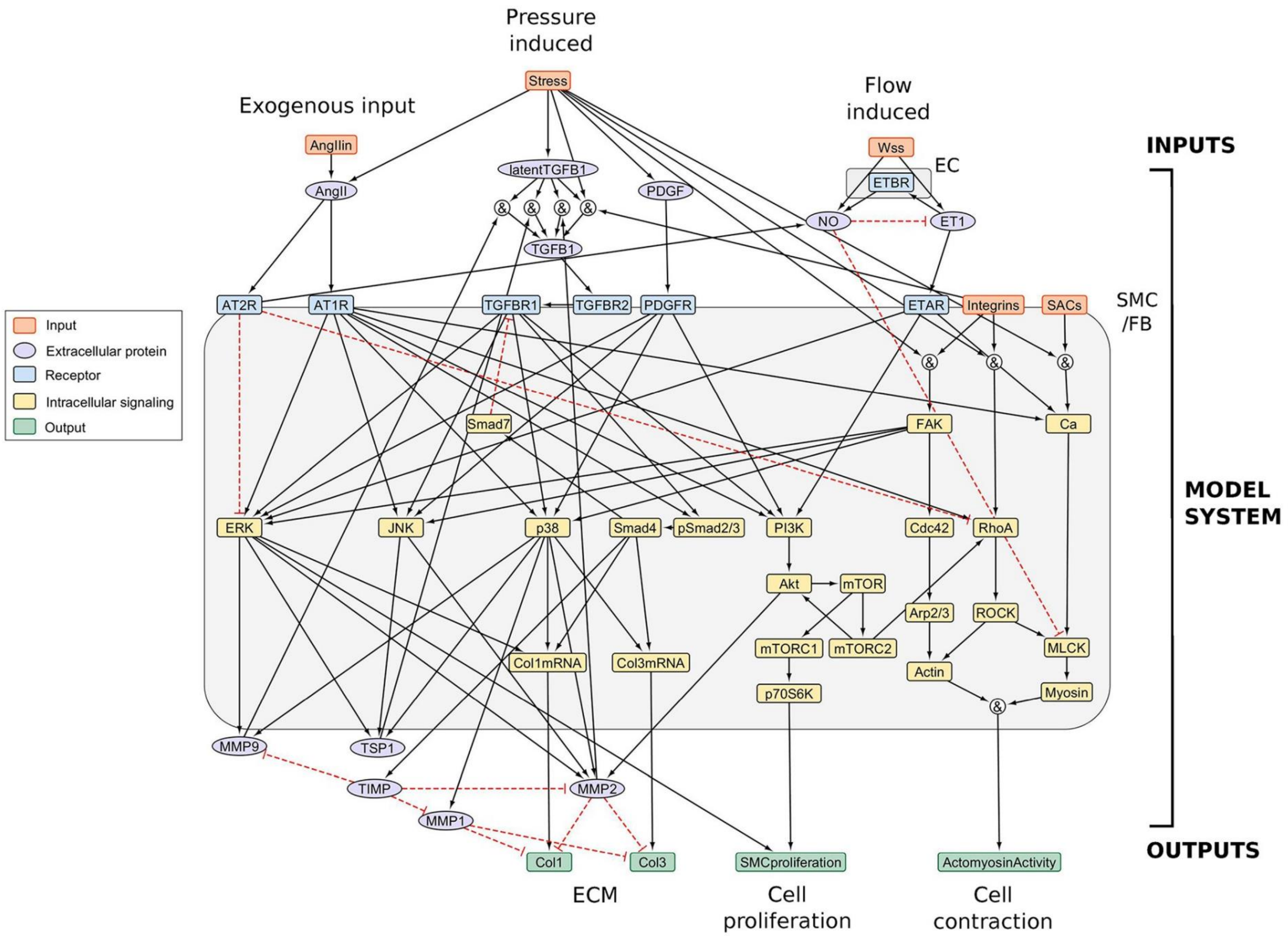
Luciano et al. Nature Physics 2021

→Cell monolayers sense substrate curvature through thickness modulation and adaptive nuclear mechanics

Life integrates all kinds of information into a complex network

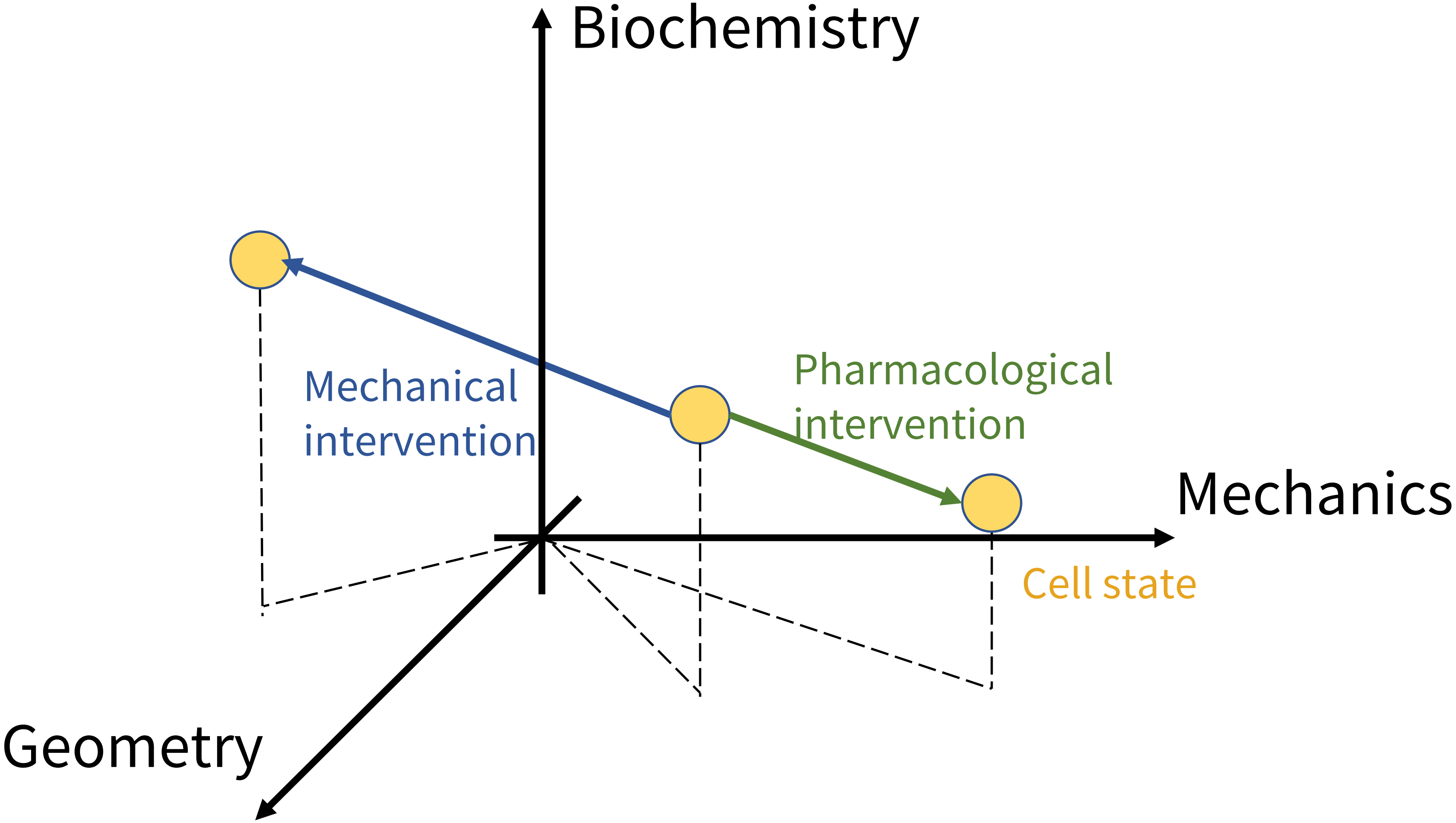


Collinet and Lecuit, Nature Reviews 2021



Irons et al., PLOS Comp. Biology 2020

The consequence of high interconnectivity for the dynamics of the network



It is impossible to uncouple these parameters

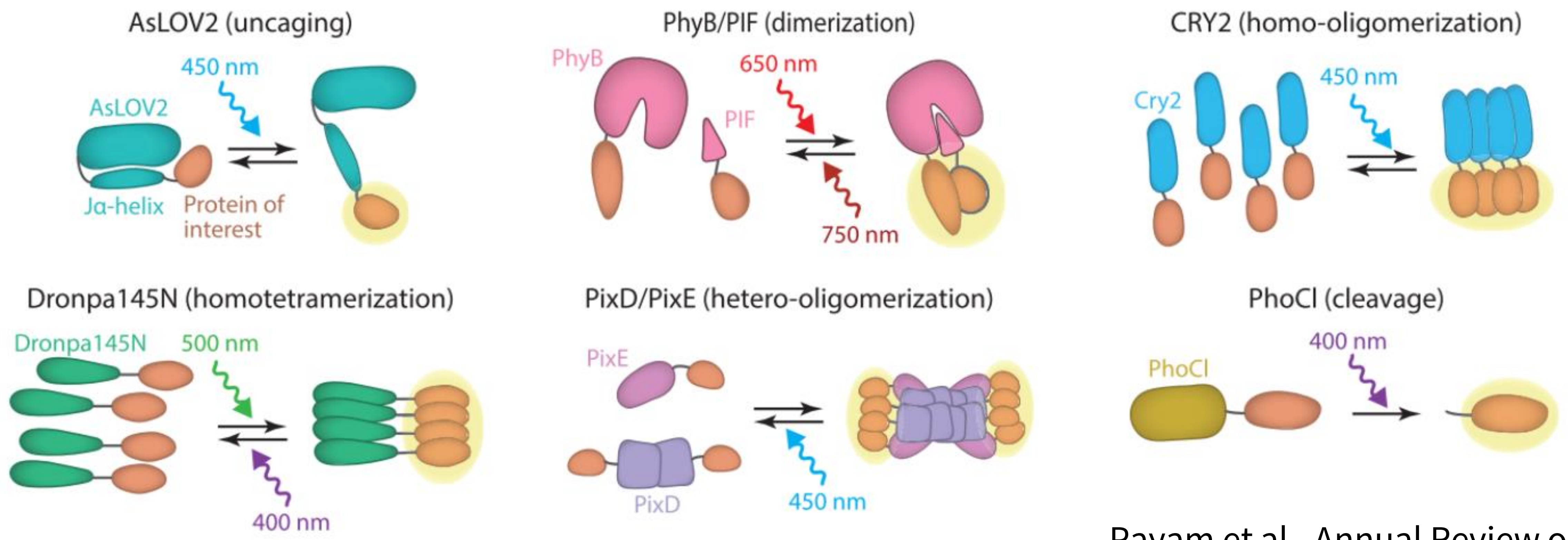
→ We need to **dynamically** control and/or monitor all of these in parallel

Problem: Most biochemical/genetical tools apply static perturbation (e.g. chemical inhibitors or knock-out experiments)

Is there a way to perturb the biochemical reaction network dynamically?

Optogenetics allows to trigger biochemical pathways with light

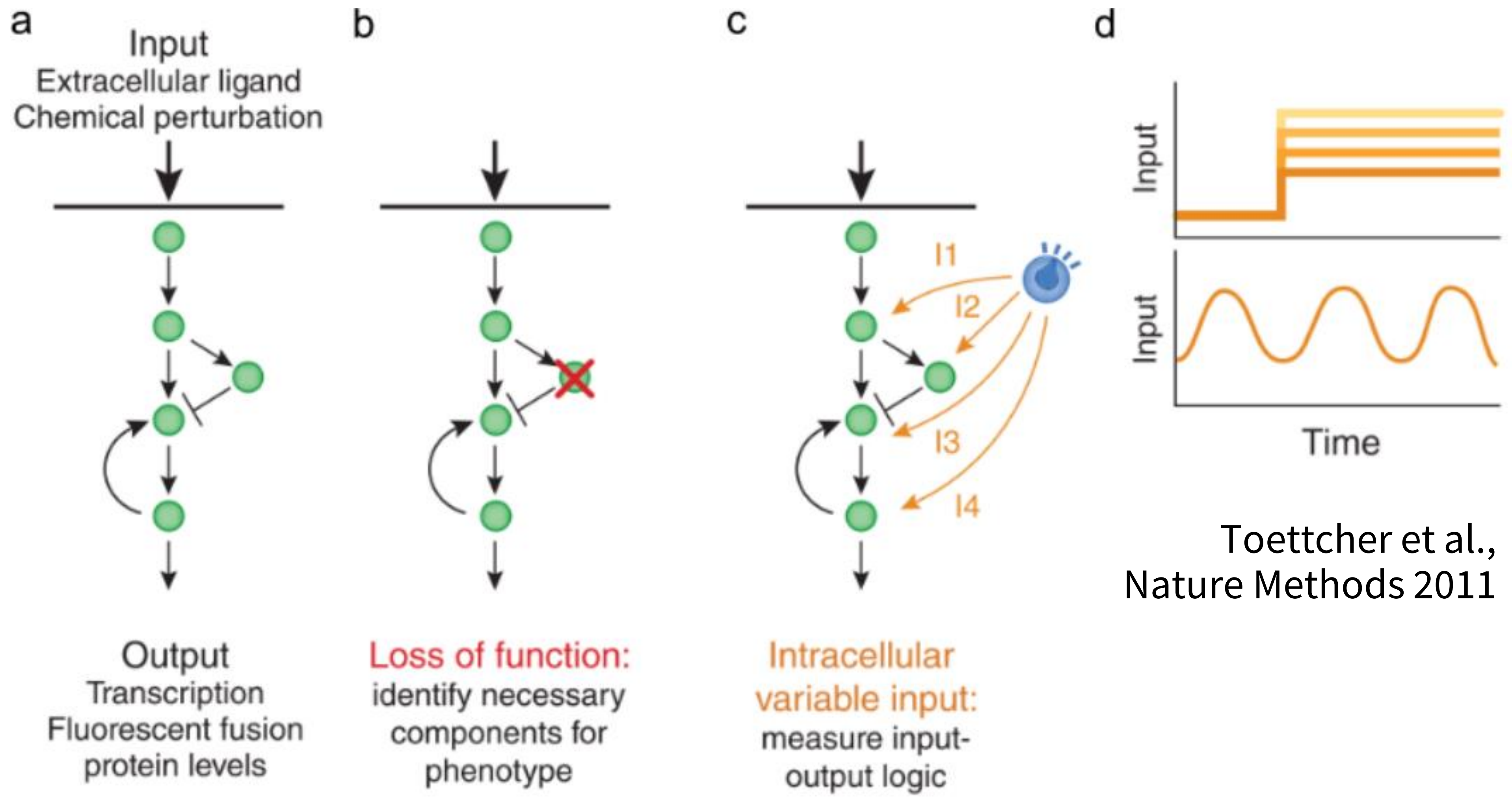
Optogenetics allows to trigger biochemical pathways with light



Payam et al., Annual Review of Biomedical Engineering 2021

Many tools have been developed since then…

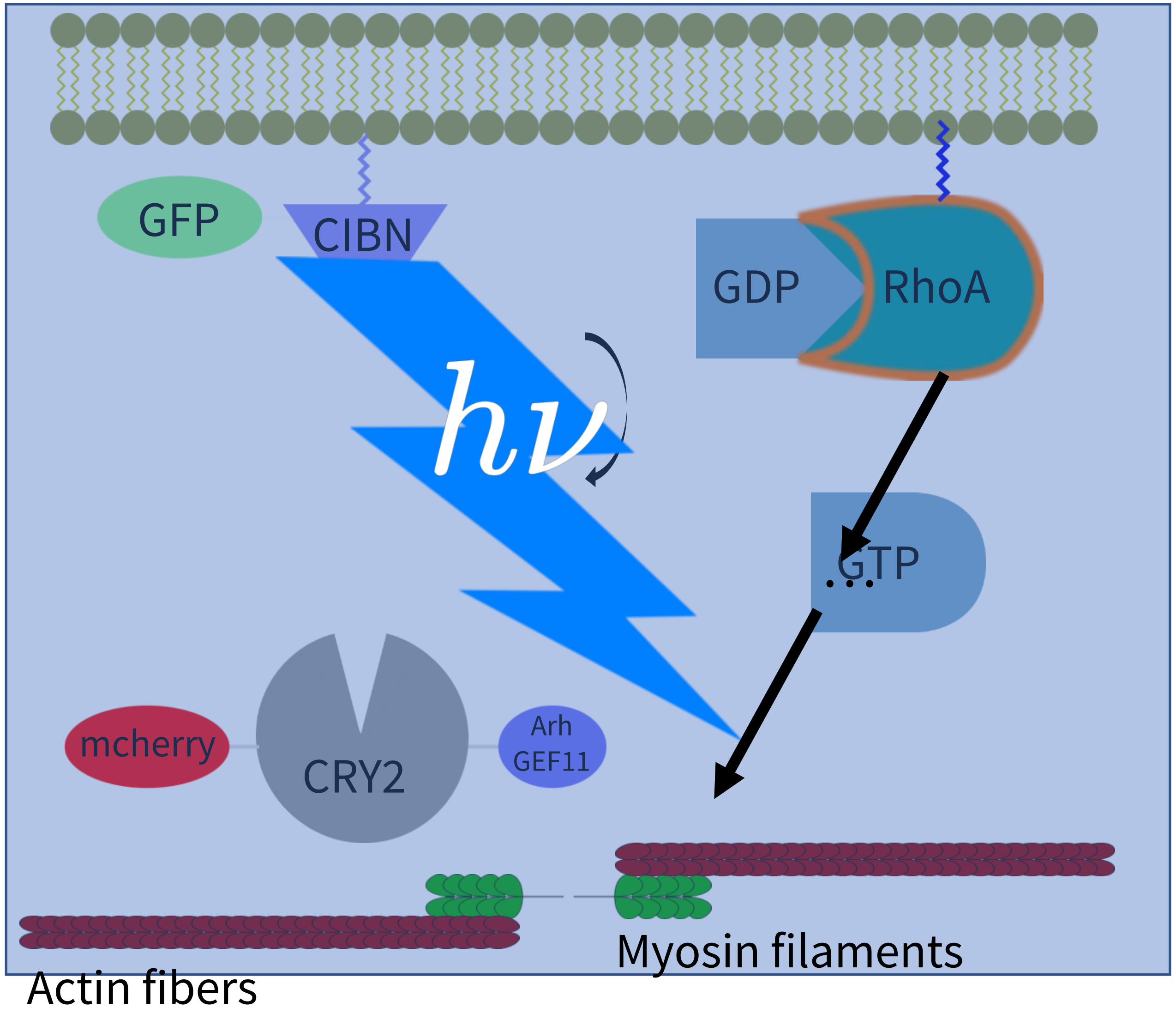
Optogenetics allows to trigger biochemical pathways with light

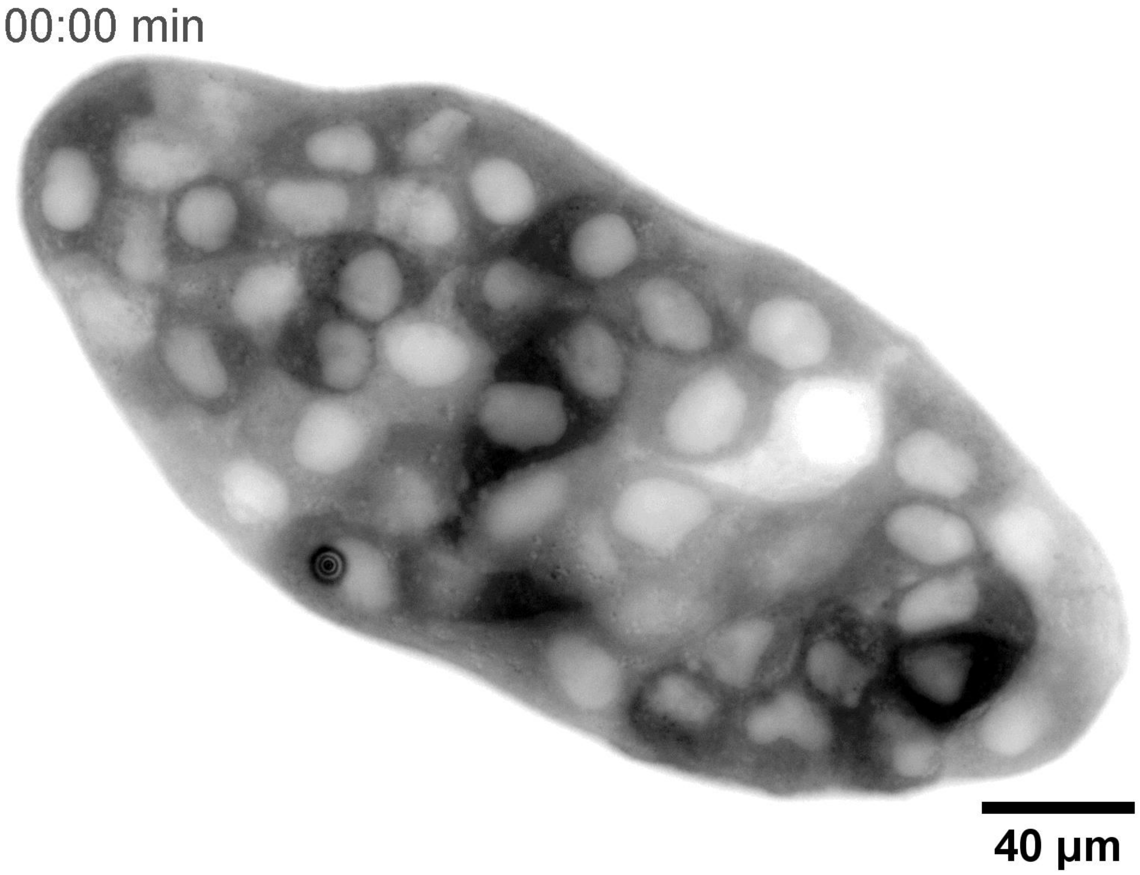


Toettcher et al.,
Nature Methods 2011

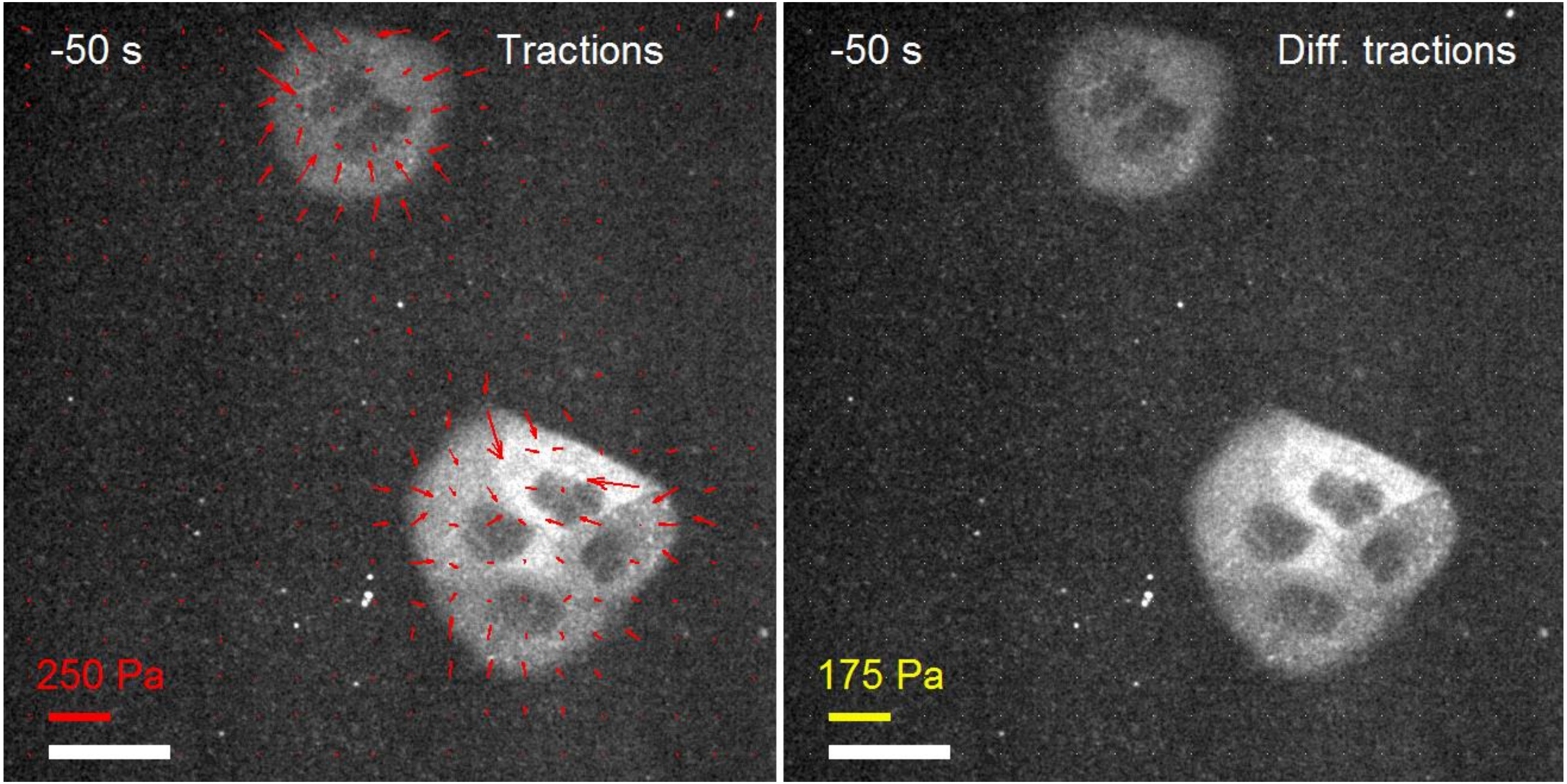
… which allow us to interrogate signaling pathways dynamically and in specific locations

Dynamic perturbartion of RhoA with optogenetics





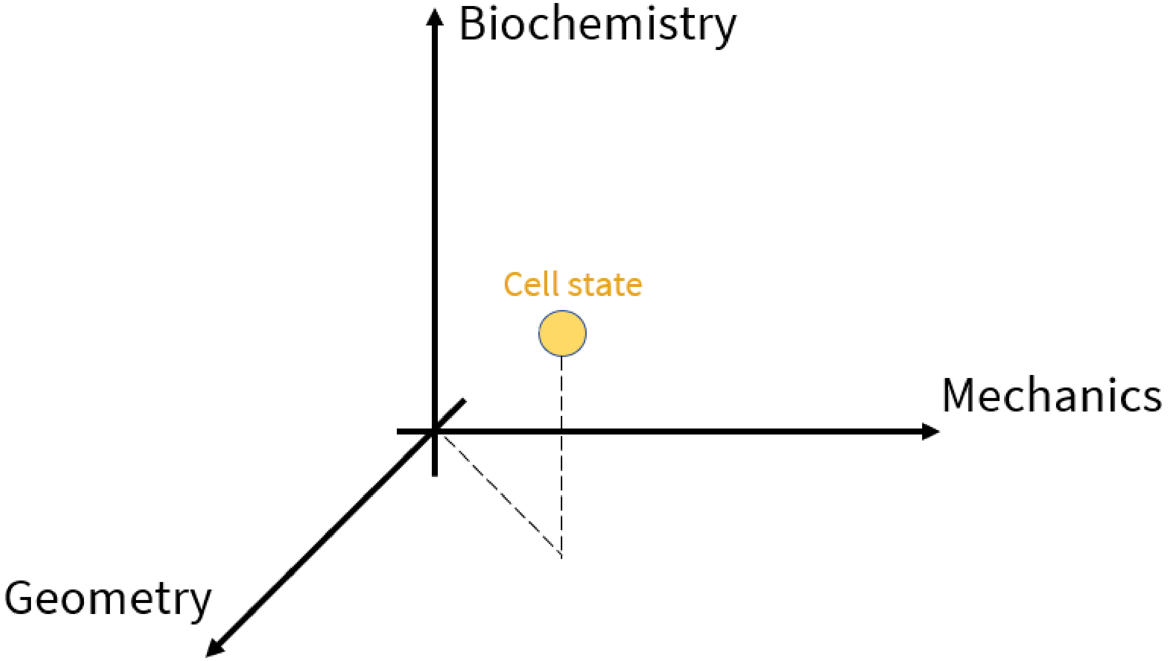
Opto-RhoA allows for dynamic and local perturbation of forces

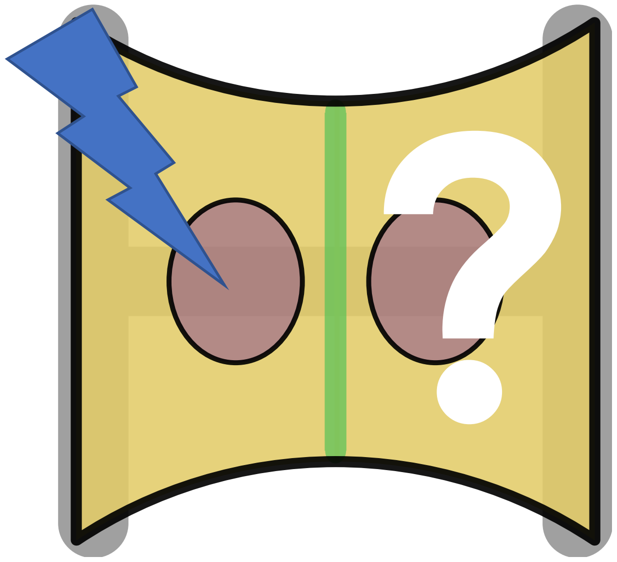


Valon et al. Nature comm. 2017

Opto-RhoA allows for dynamic and local perturbation of forces

With optogenetics, we can generate dynamic and local RhoA signals.
→How do these signals get perceived by another cell?
→How does this depend on cell geometry?



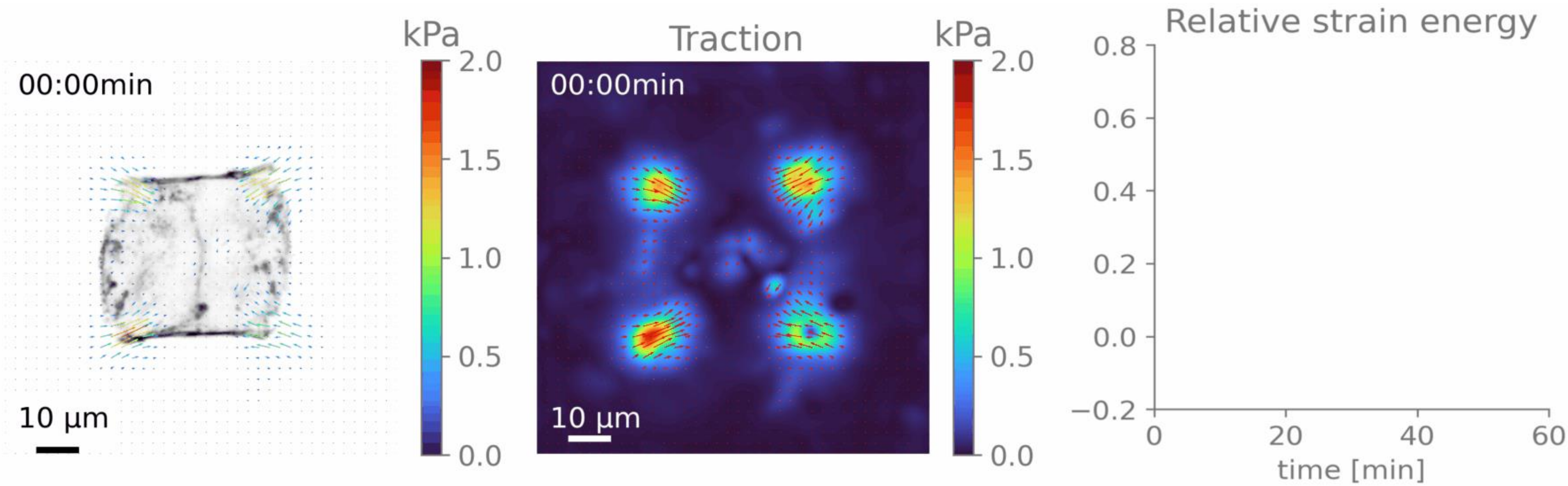


→Use micropatterns to get cell doublets of controlled shape, optogenetics to create local and dynamic RhoA signal and TFM as a dynamic force readout

Force signal propagation in cell doublets

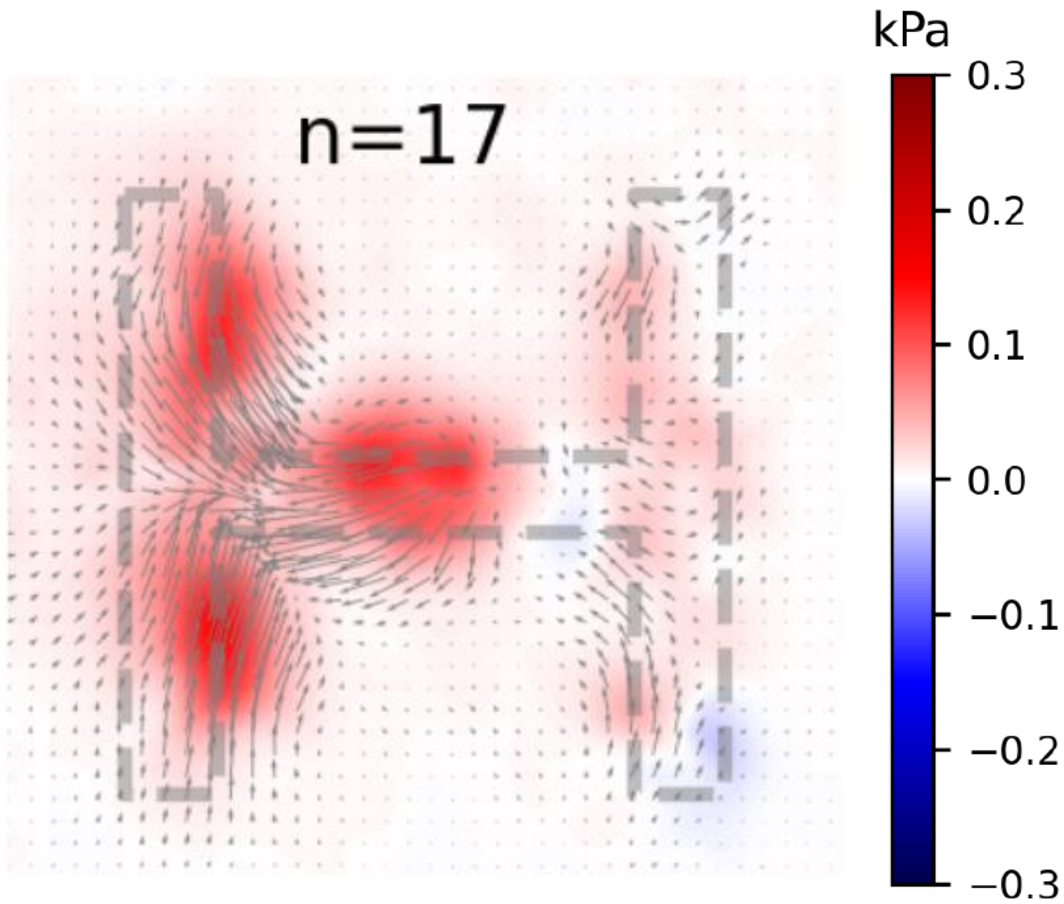
Manasi Kelkar

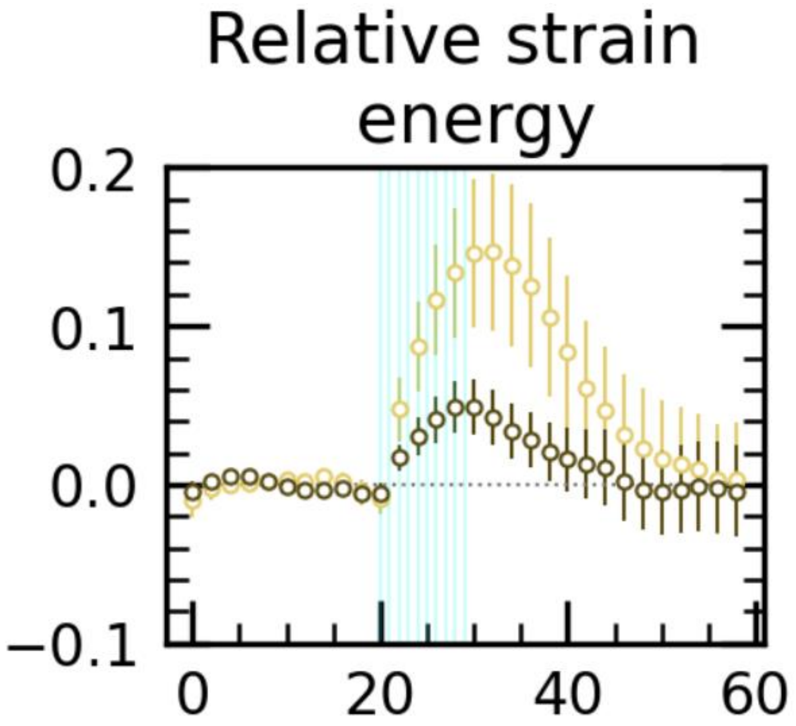
Guillaume Charras

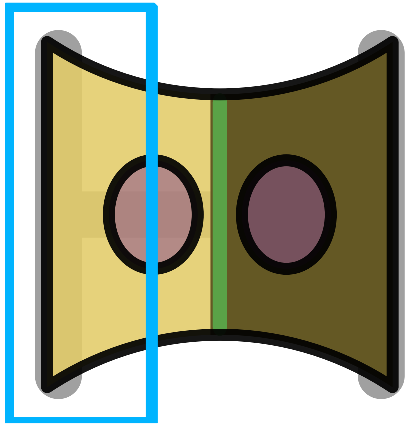


Left half Right half

Force signal propagation in cell doublets





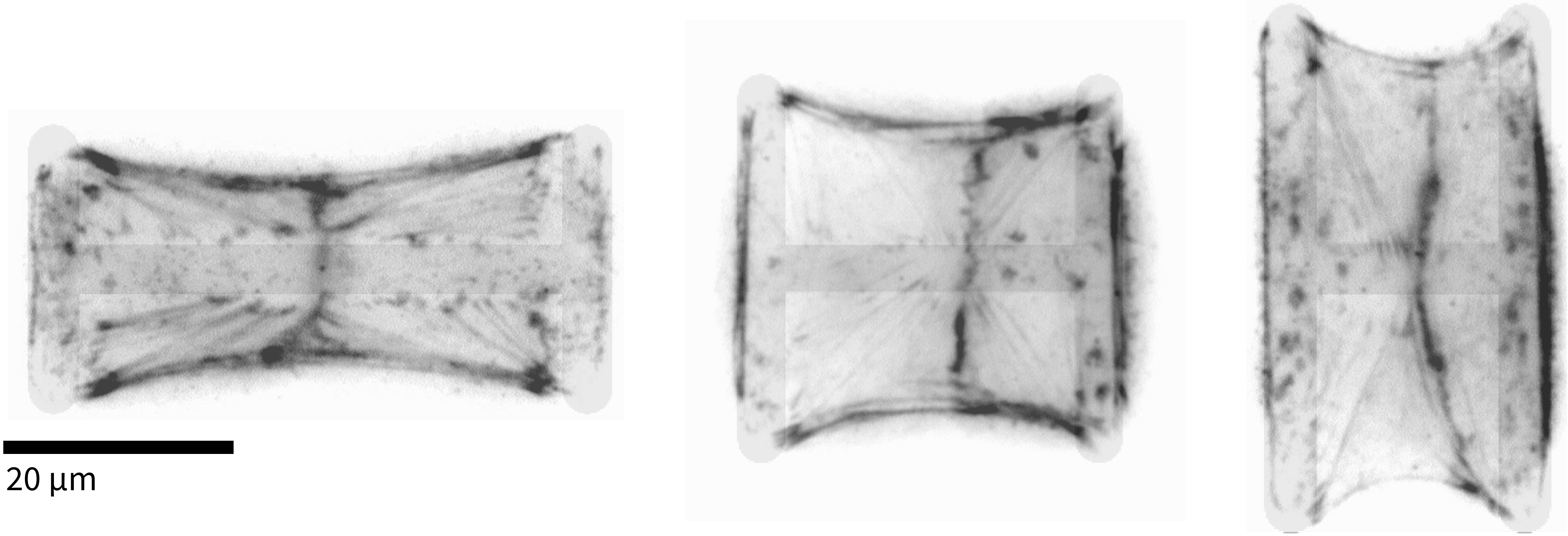


Force increase Force decrease

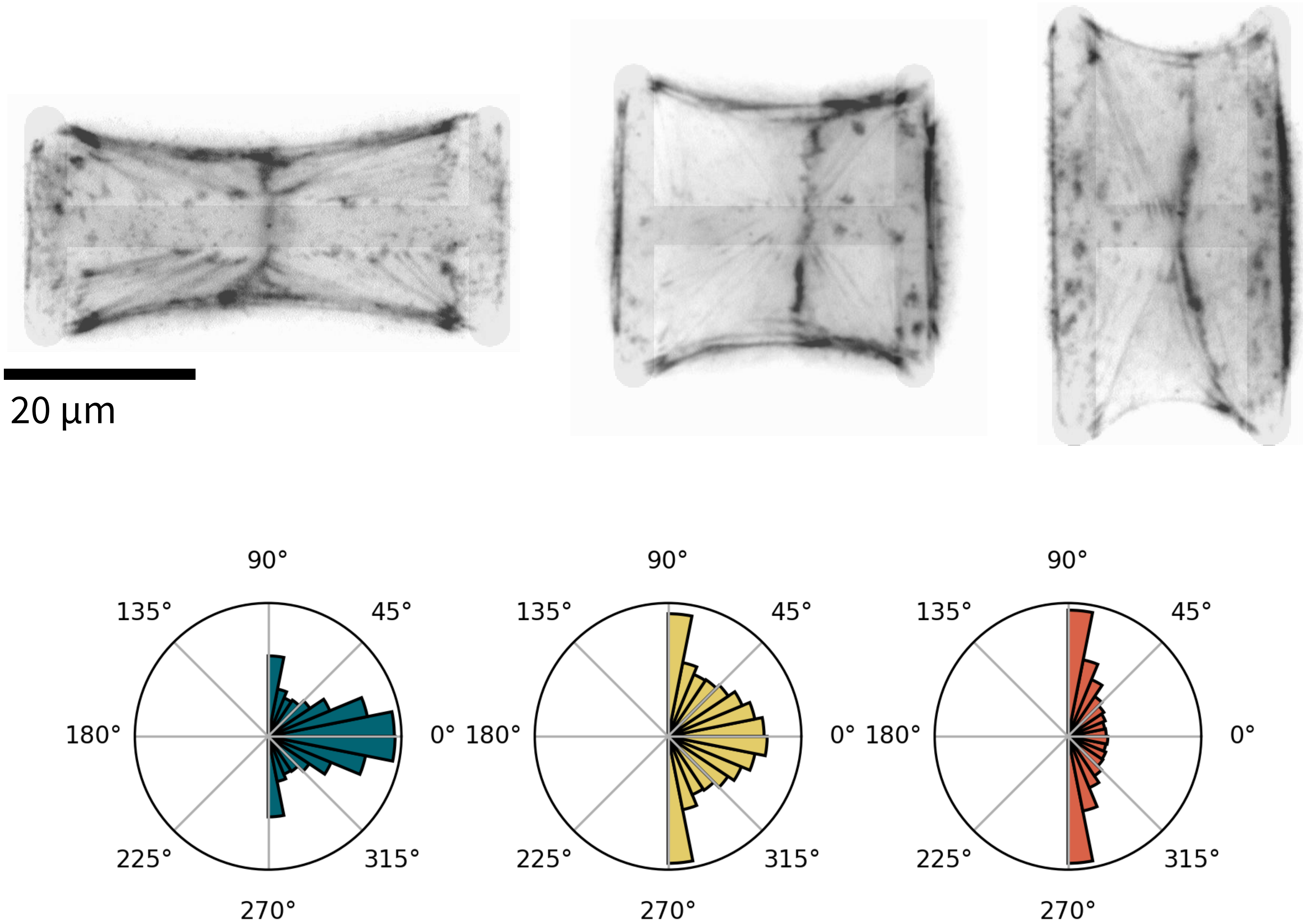
→ Non-activated cell contracts as well, but less strongly

Micropatterns to vary the geometry of the doublets

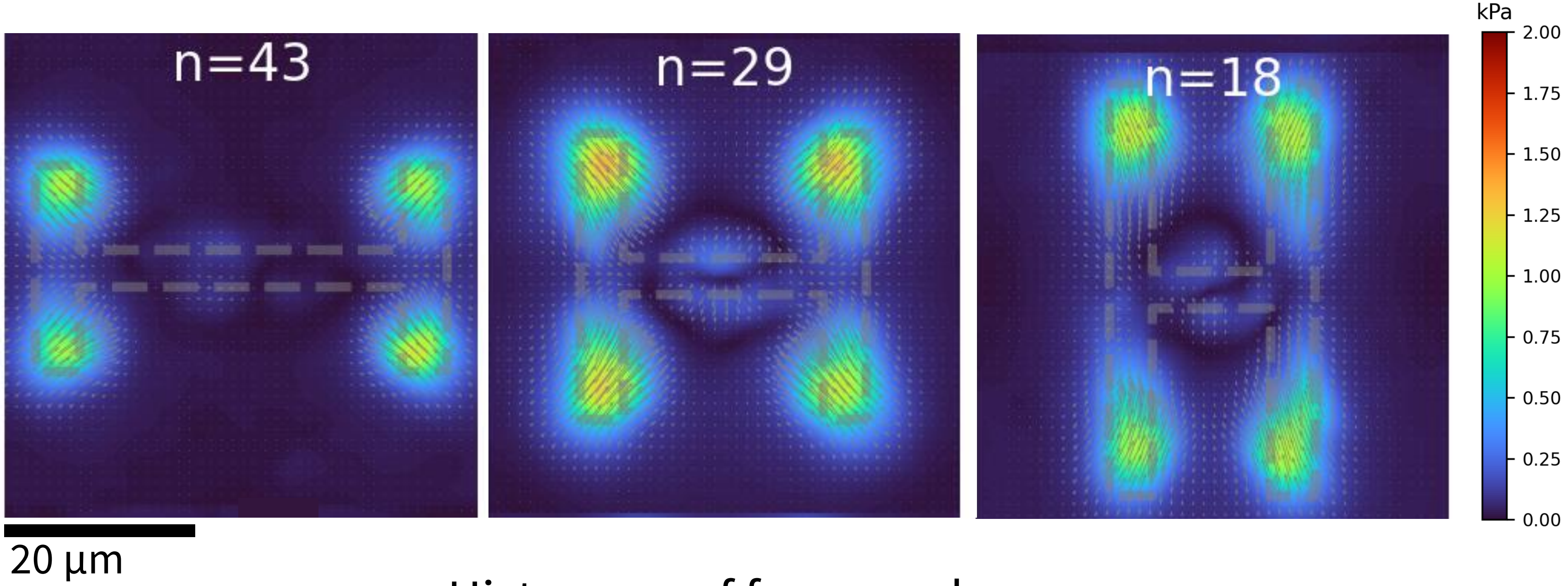
Micropatterns to vary the geometry of the doublets



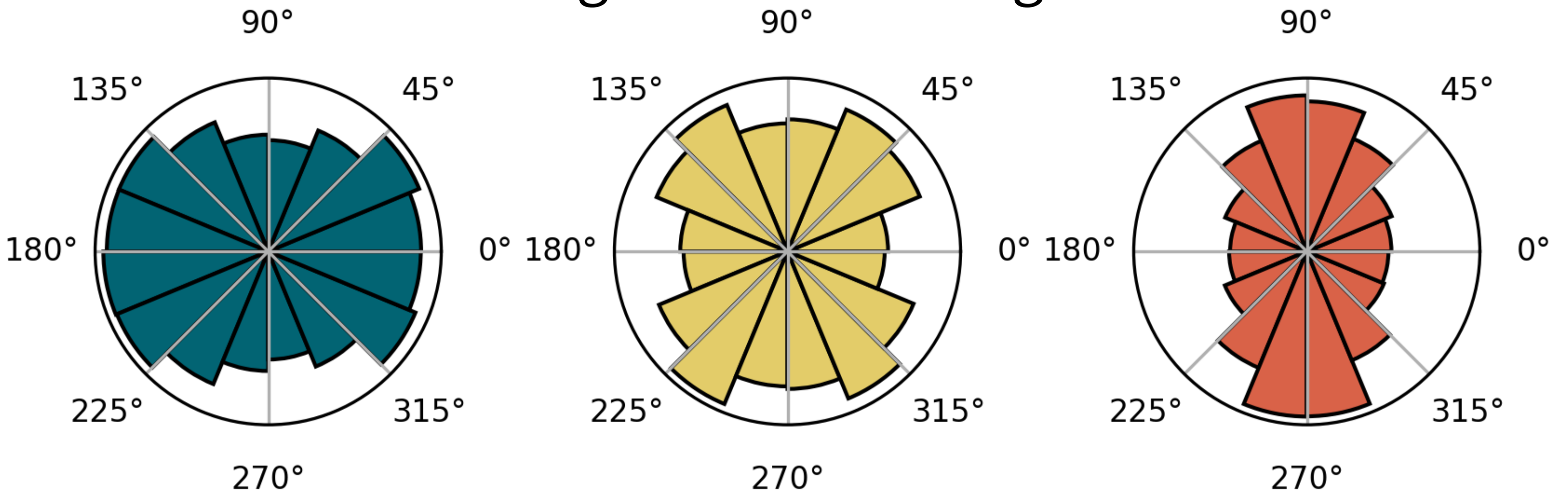
Change in cell shape leads to change in mechanostrutcutral polarization



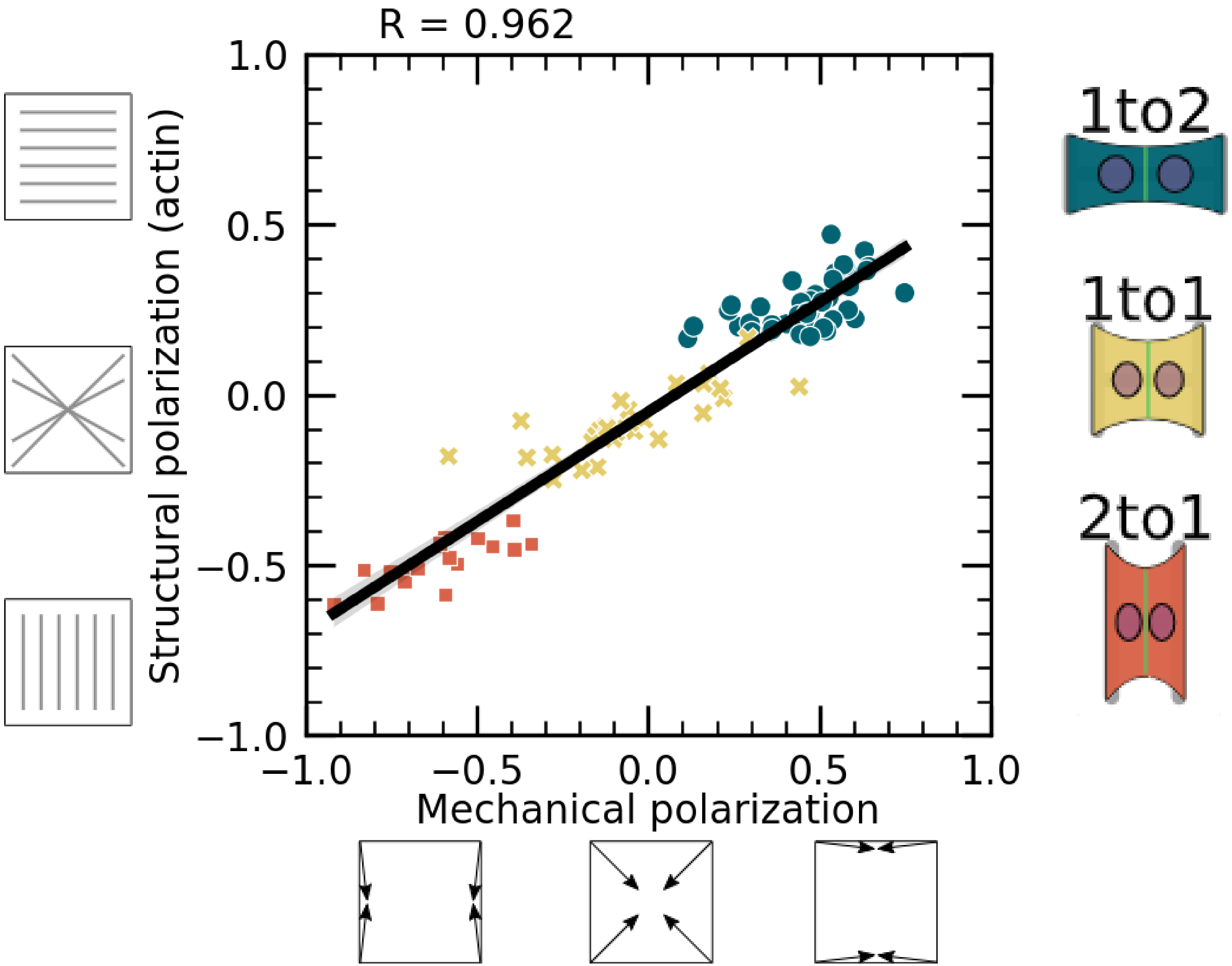
Change in cell shape leads to change in mechanostrutcutral polarization



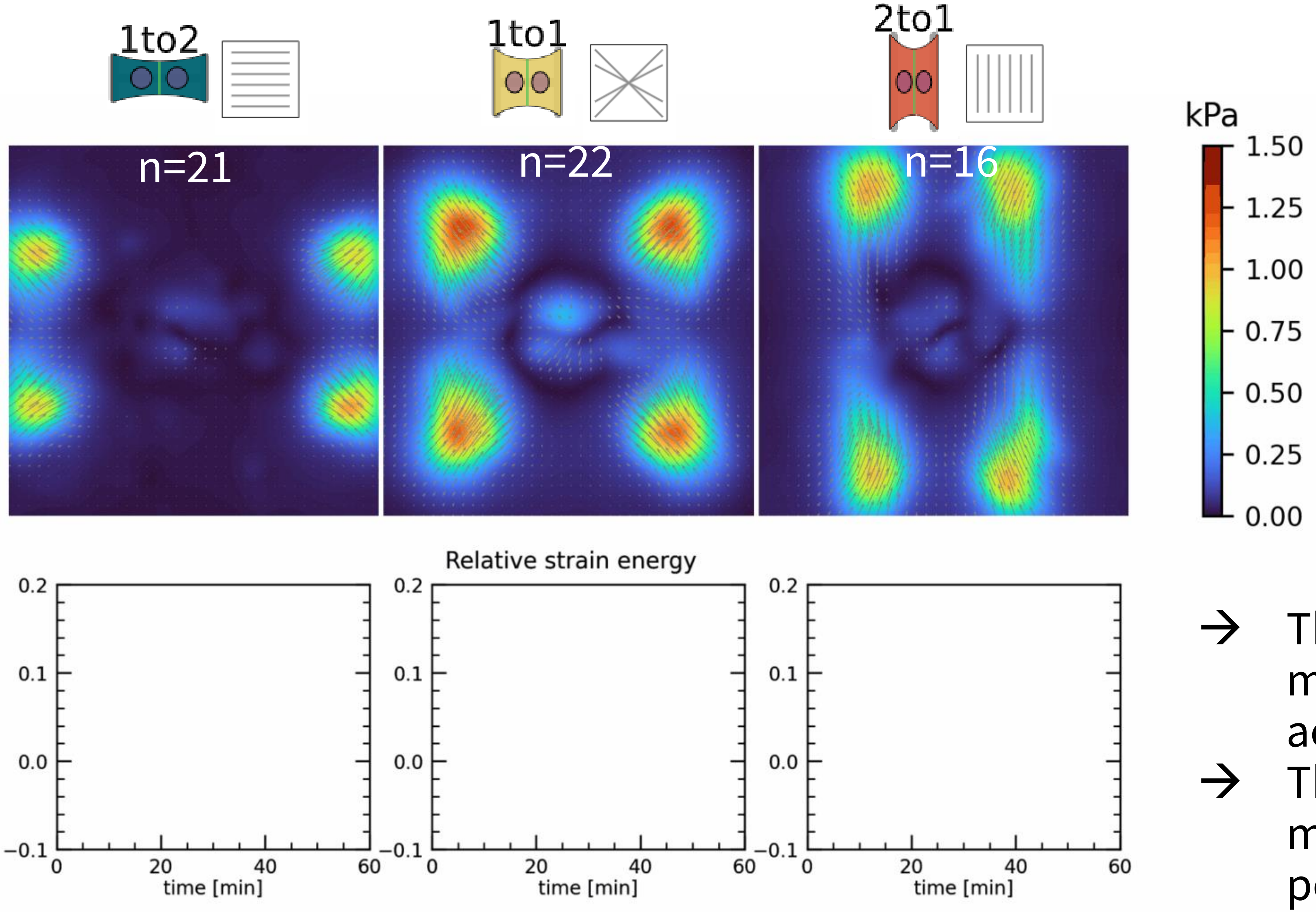
Histogram of force angles



Change in cell shape leads to change in mechanostrutcutral polarization



Influence of mechanostructural polarization on force signal propagation



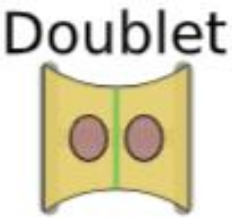
- → The non-activated cell is mechanically coupled to the activated cell
- → This coupling is related to the mechanostructural polarization of the doublets

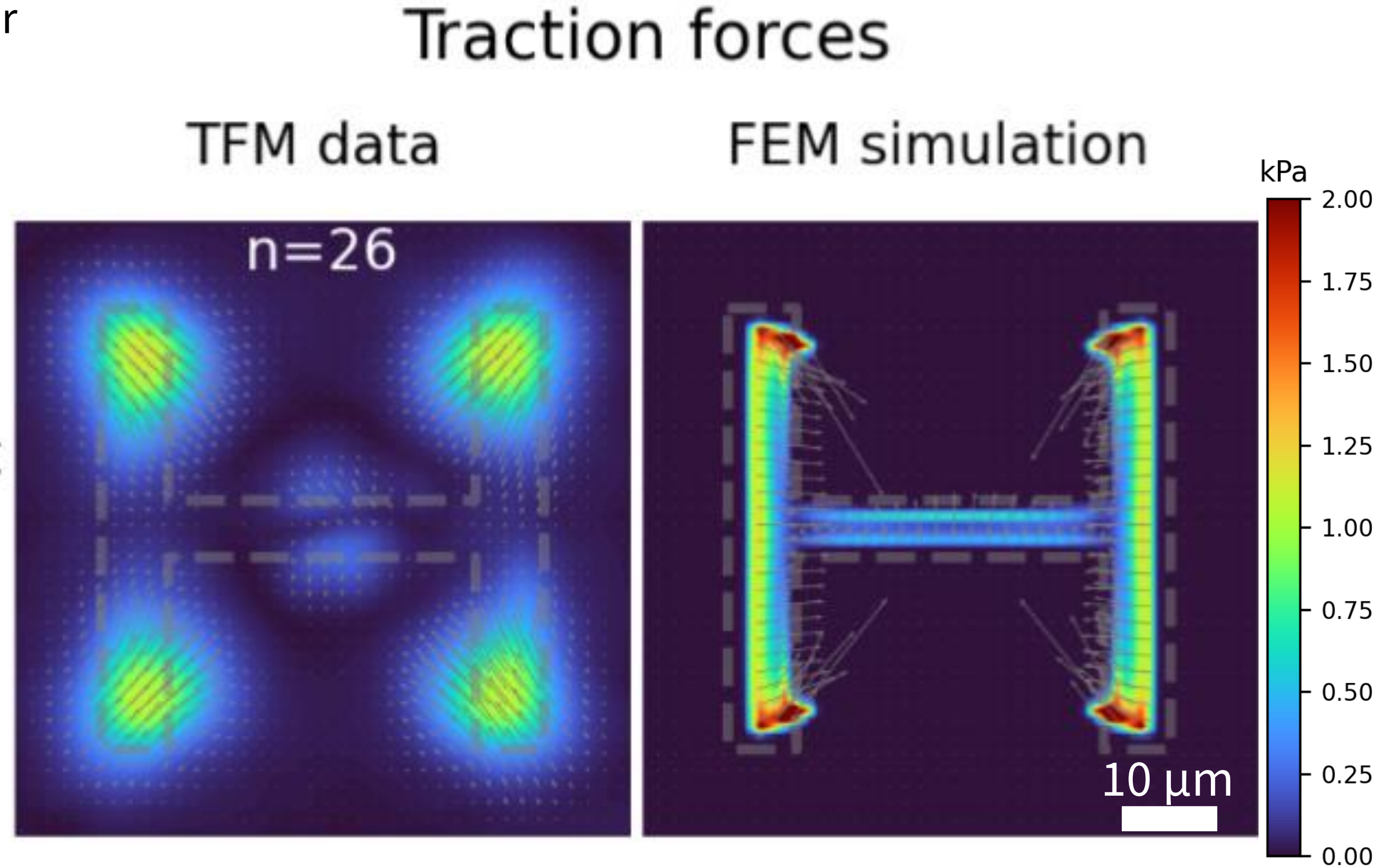
Is the response of the non-activated cell active or passive?

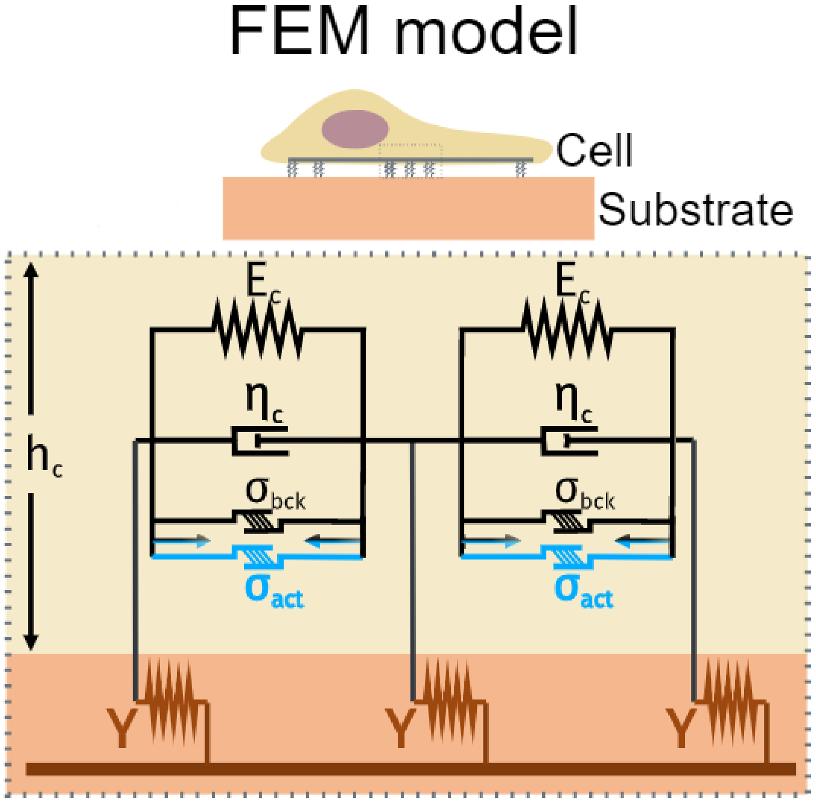
Comparing experimental data to passive elastic model

Dennis Wörthmüller

Ulrich S. Schwarz







Comparing experimental data to passive elastic model

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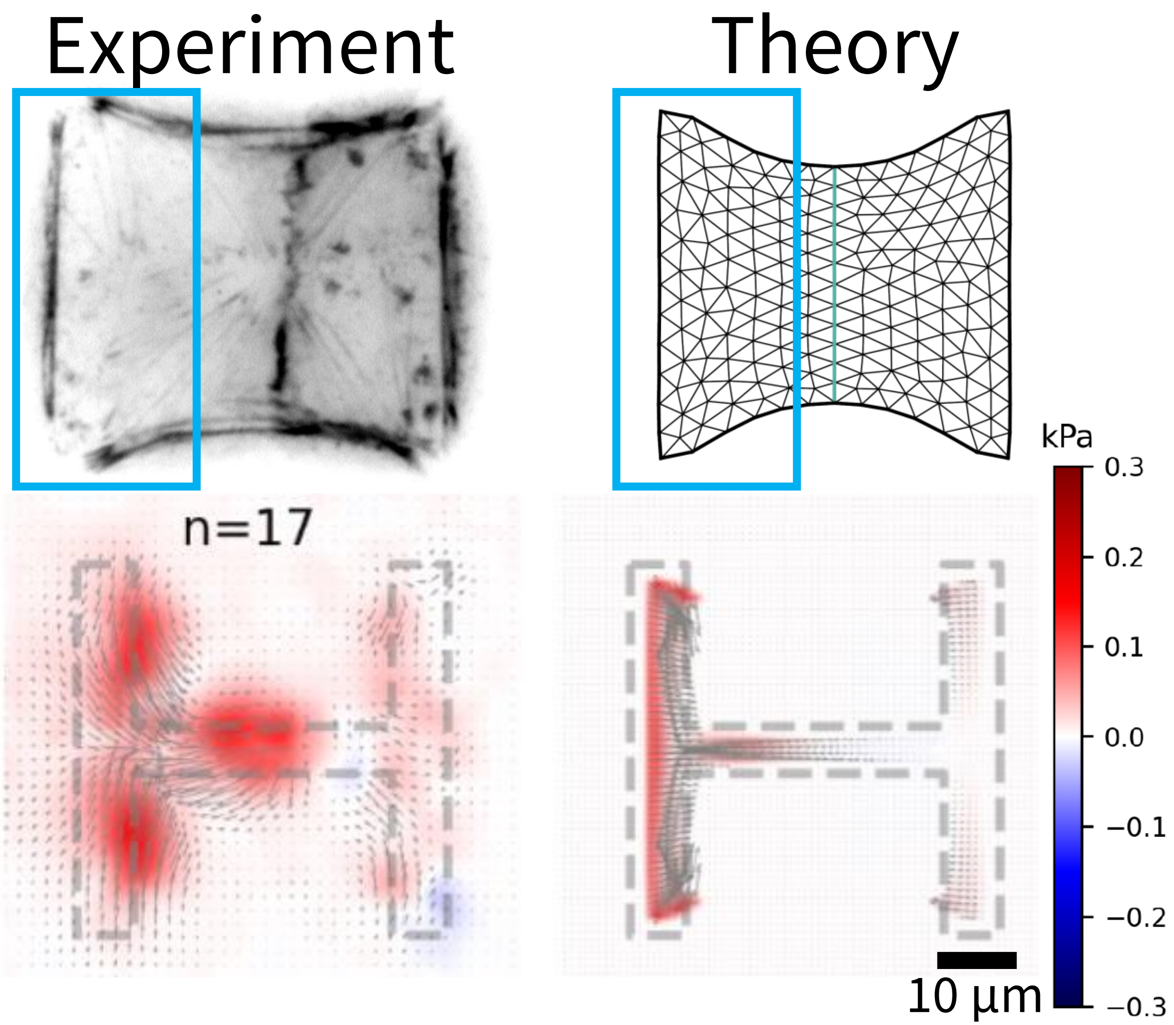
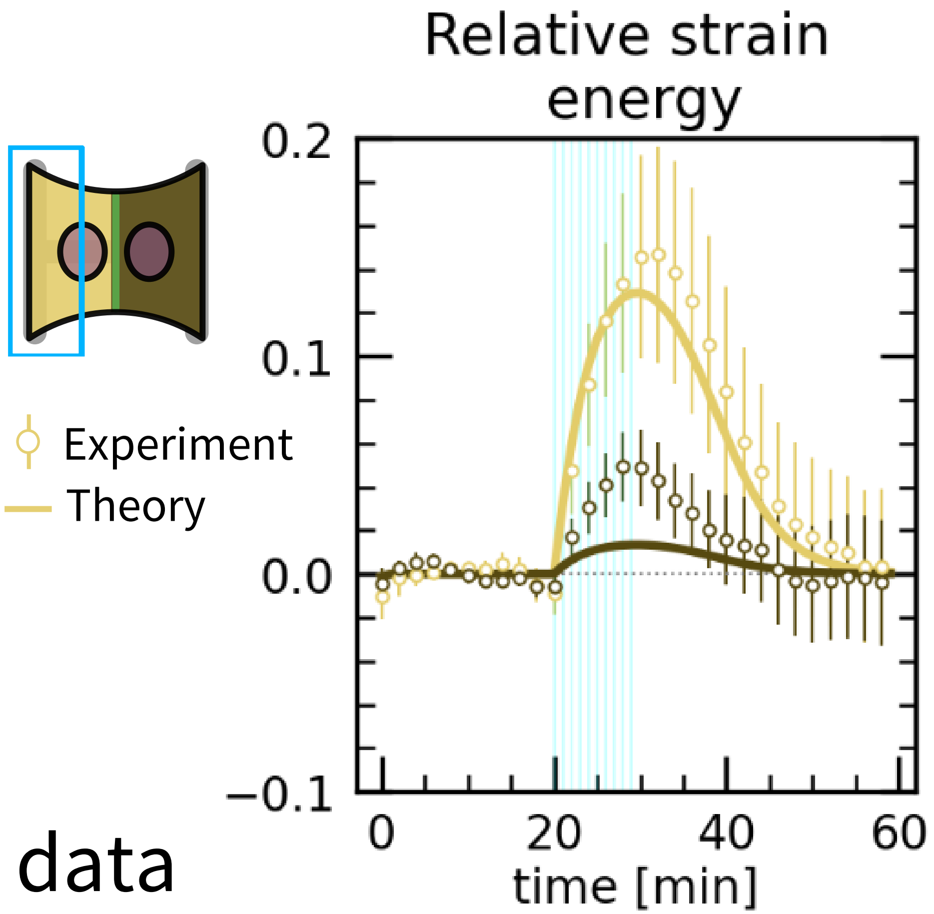
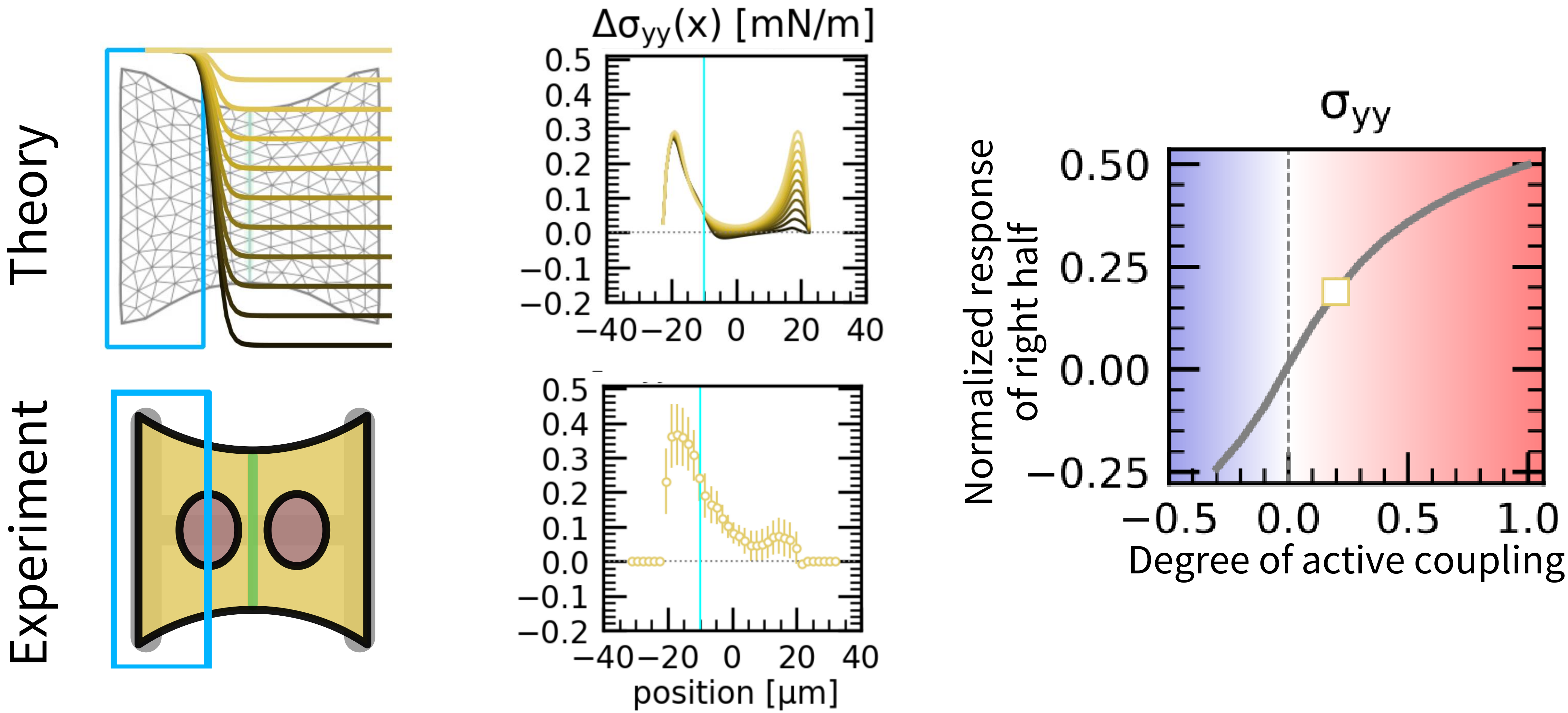




→Purely passive model doesn't account for data

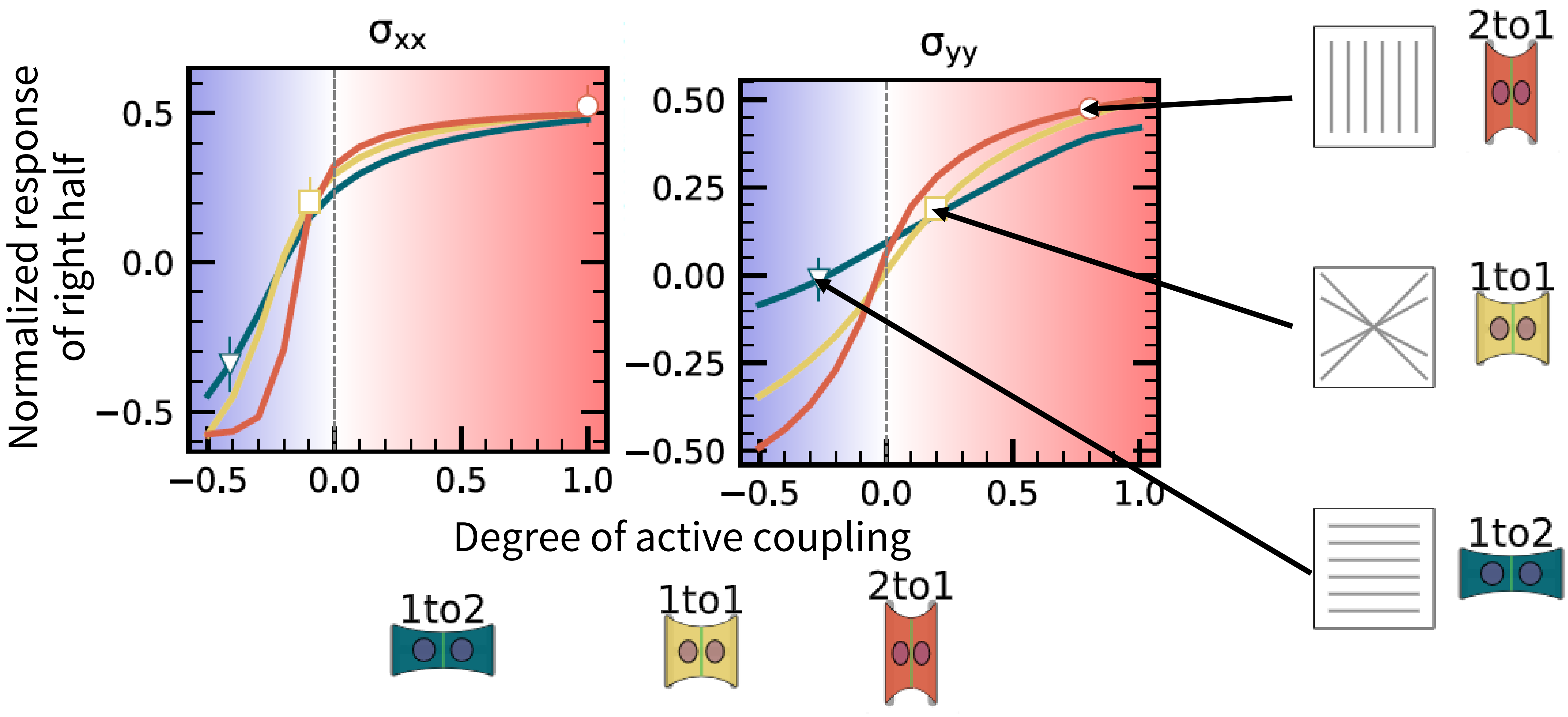
→Add active coupling term to model

Implementing active coupling



→Non-activated cell reacts actively

Active coupling analysis



→Active coupling is tightly linked to mechano-structural polarization

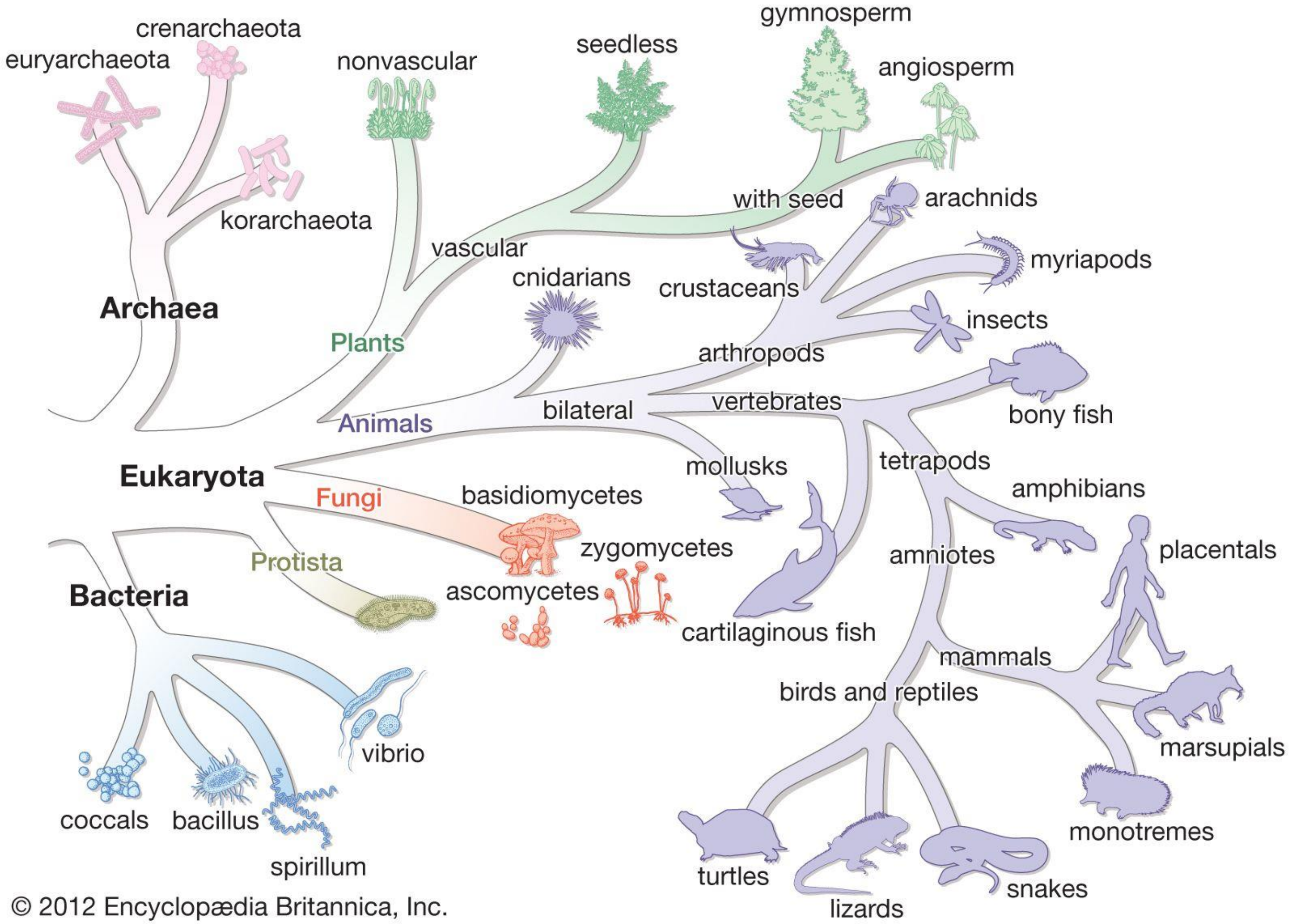
Conclusion

→ Cells respond actively to the contraction of a neighbouring cell
→ We can quantify this active response by combining TFM, optogenetics and mathematical modelling
→ This active response is modulated by the mechano-structural polarization of the cell

→ **Stress propagates actively in cell doublets**
→ **Stress propagates preferentially perpendicular to the polarization axis**

Take-home-message

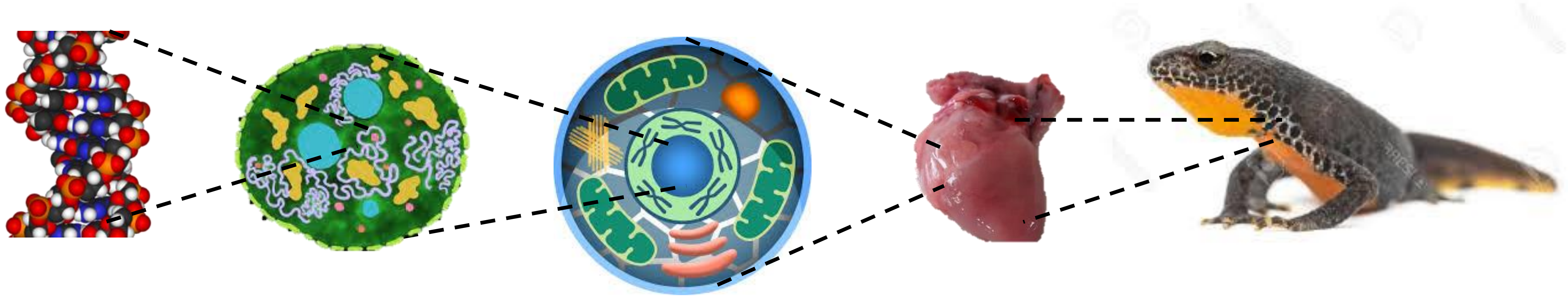
Take-home-message



"Nothing in Biology makes sense except in the light of evolution"
Theodosius Dobzhansky

Through the evolutionary process, life accumulated complexity and information
This makes every species, even every individual, unique, but connected through history to all other life forms
→ To understand life, we need to study its evolutionary history

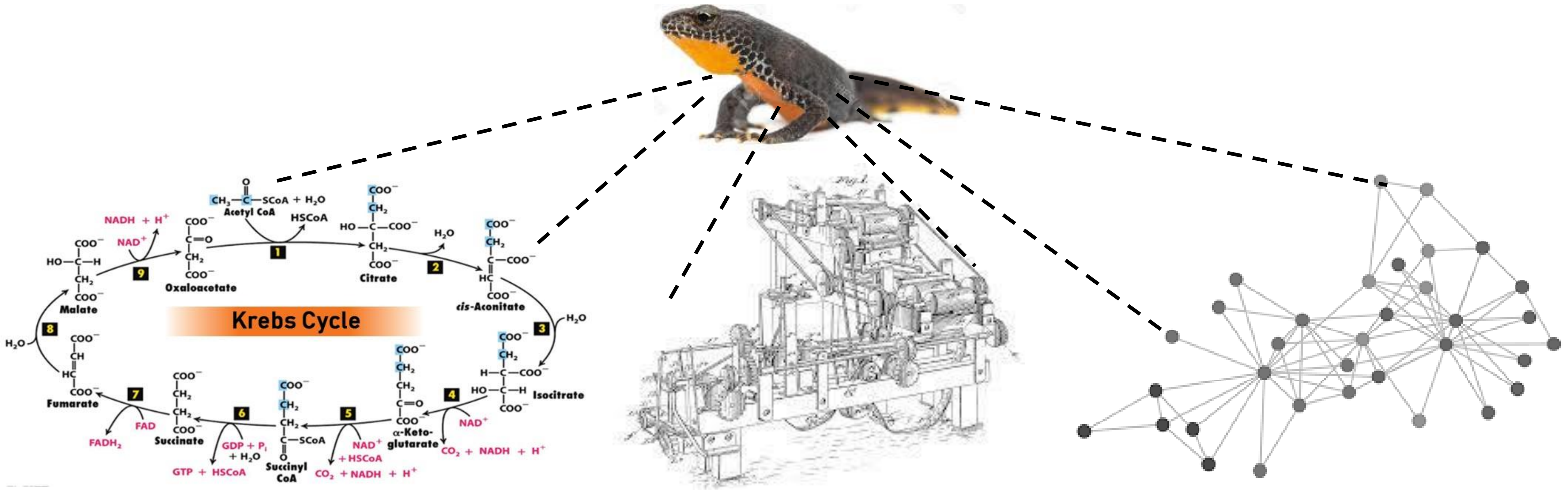
Take-home-message



Living systems are layered, complex architectures spanning multiple length scales.
At each layer, the highly connected parts lead to new, emerging properties and behavior

→ To understand life, we need to study it at all scales

Take-home-message



The networks at each layer contain geometrical, mechanical, chemical, electrical, (…), information which all influence each other

→ To understand life, we need to study it from different perspectives (chemistry, physics, information theory, …)