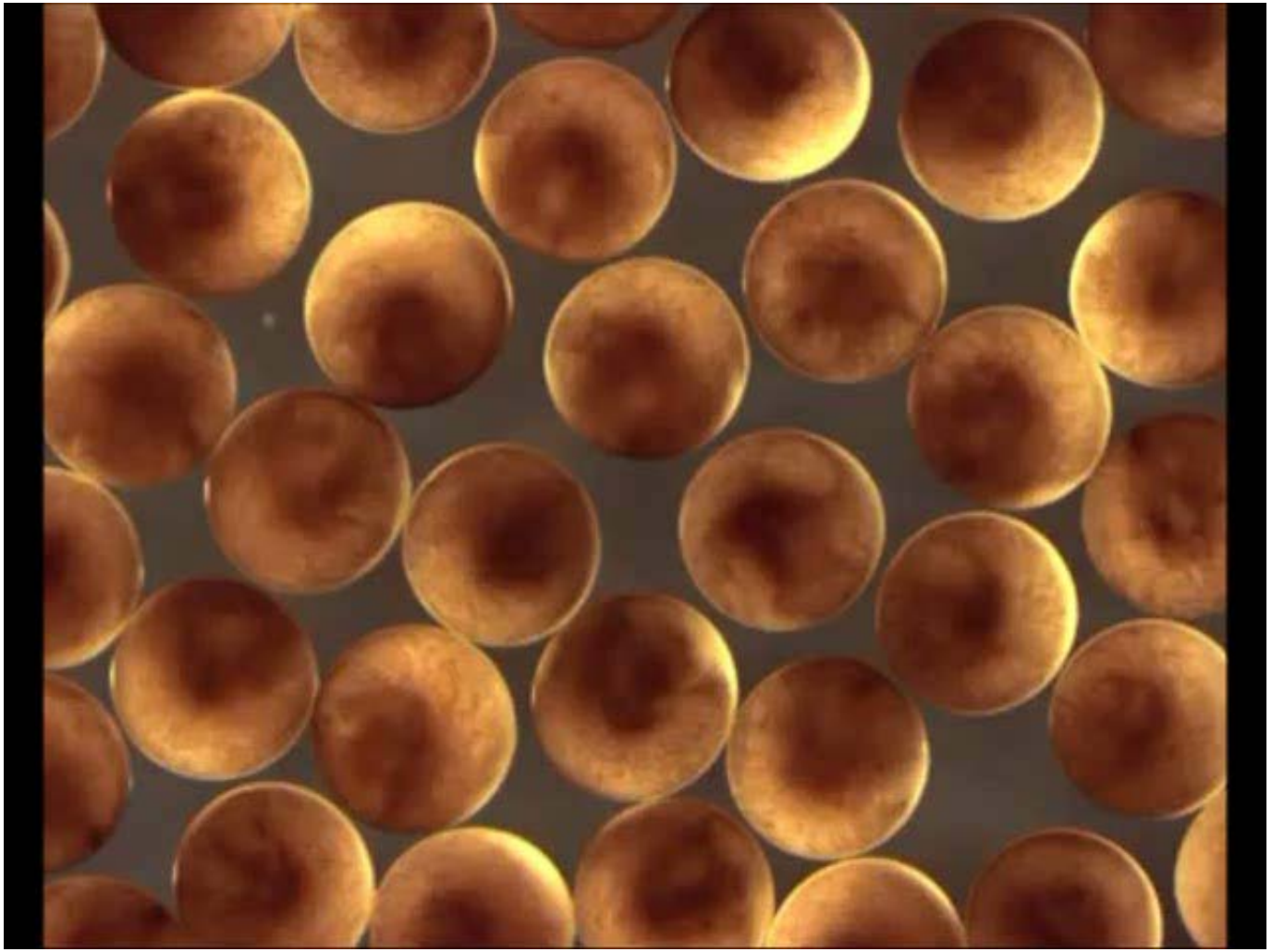
Alive or dead?

Self-organization of biological structures

Dan Fletcher
UC Berkeley / LBNL

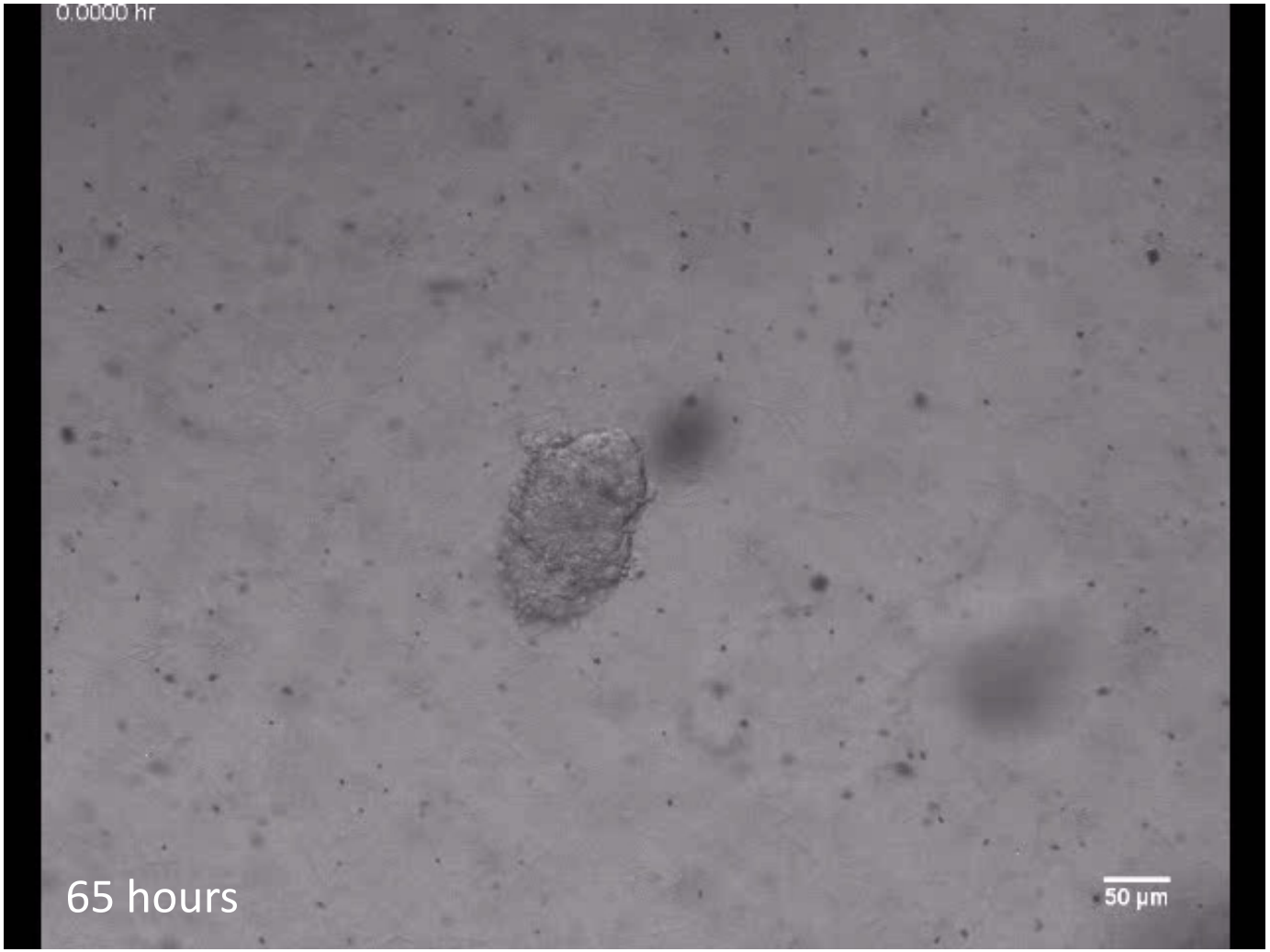
Development



H. Williams and J. Smith

Xenopus laevis

Morphogenesis



G. Venugapalan and D. Fletcher

Mouse mammary epithelial cells (EpH4)

Self-organization

Formation of global order out of a series of local interactions, involving energy consumption.

Properties of living systems:

- Out of equilibrium
- Robust, adaptive
- Self-healing, self-scaling
- Sense & respond to external signals
- Programmed by molecules

Can these properties be harnessed by materials?
Can self-organized biological structures be built?

Building biological structures

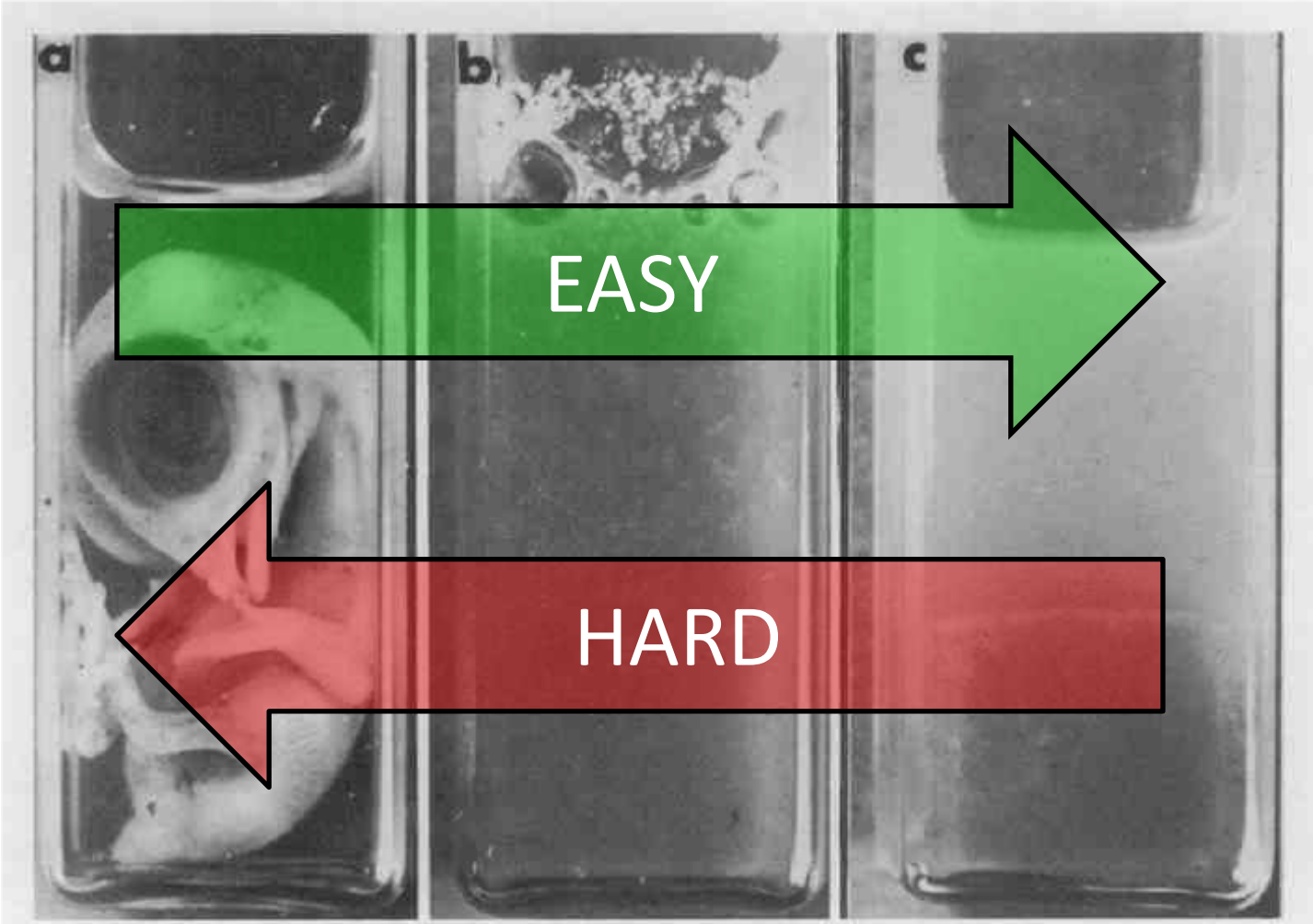
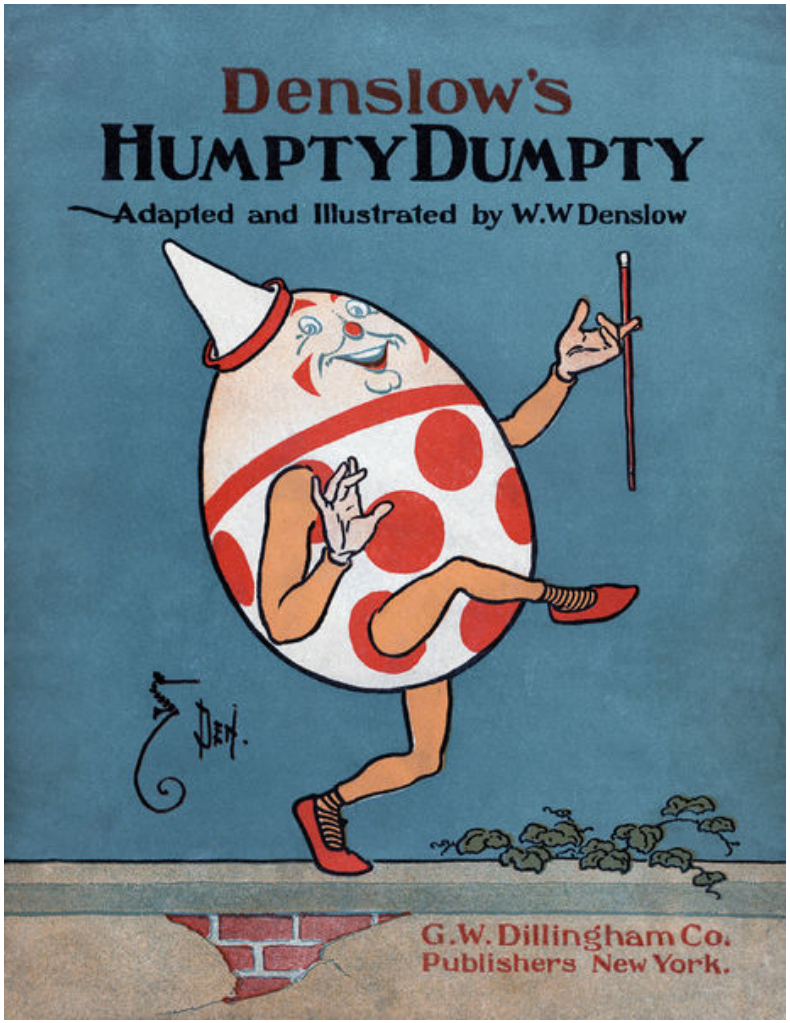


FIG. 2. Chick embryo (*a*) intact (*b*) homogenized (*c*) fractionated. (*Original.*)

Paul Weiss, 1962

Chick embryo

A childhood warning…



W. W. Denslow, 1904

Humpty Dumpty sat on a wall,
Humpty Dumpty had a great fall.
All the king's horses and
all the king's men
Couldn't put Humpty together again.

c. 1787

…but some still try

Mary Shelley, 1918 *Frankenstein*

Why attempt to rebuild biology?

The goal: Understand the physical and chemical mechanisms that govern assembly and function of biological structures

The approach: Bottom-up construction of cellular structures, starting with molecules (proteins, DNA, lipids, etc) – known as 'cellular reconstitution'

The use: Create materials with unique properties, such as the ability to sense and respond and the ability to self-repair

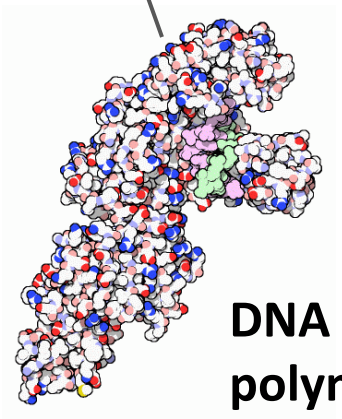
A brief history of reconstitution

Timeline | **Examples of biochemical and cellular reconstitution**

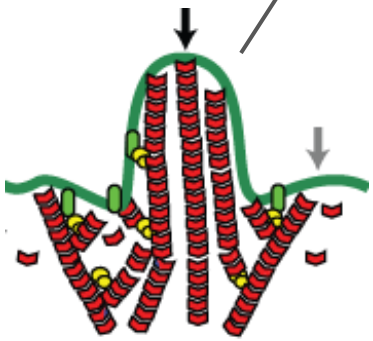
Liu & Fletcher, *Nat. Rev. MCB*, 2009

- 1942–1950: Characterization of ATPase activity of actomyosin[6]
- 1956–1960: Discovery and biochemical reconstitution of DNA polymerase activity[8]
- 1978: Enzymatic activity of DNA gyrase[13]
- 1984: Protein transport within Golgi stack[11]
- 1992: Polypeptide translocation in endoplasmic reticulum[14]
- 1996: Bipolar spindles in *Xenopus laevis* extract[20]
- 1999: Hybrid immunological synapse[31]; Actin-based motility using purified proteins[22]
- 2003: Filopodium-like bundles from dendritic networks[24]
- 2007: Bacterial plasmid DNA segregation[27]
- 2008: Filopodia-like protrusion on giant vesicles[48]; Filamenting temperature sensitive mutant Z (FtsZ) alone found to cause constriction[50]; Travelling waves of MinD and MinE[33]; Dynamin alone found to be sufficient for membrane fission[35]
- 2009: ESCRTIII (endosomal sorting complex required for transport III) found to cause membrane budding and scission[67]

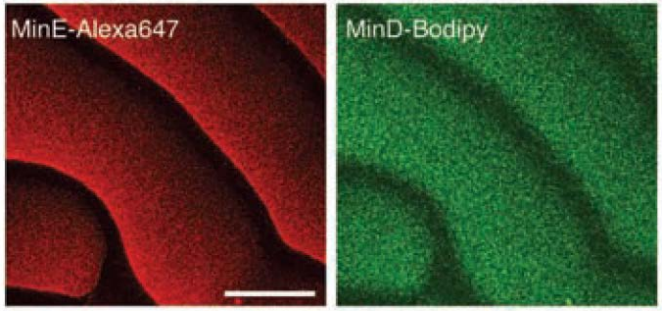
Biochemical reconsitution on a molecular-length scale (red) and cellular reconstitution on a micrometre scale (black).



DNA polymerase



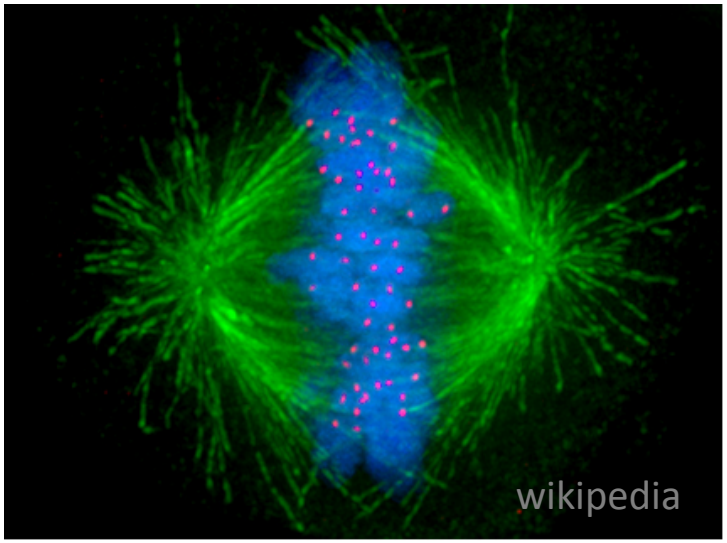
filopodia



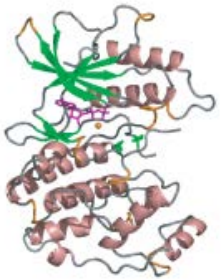
Min DE waves

The spindle

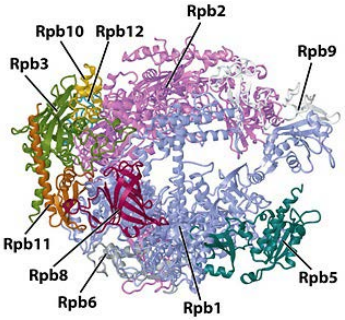
A microtubule-based structure (green) that organizes and separates chromosomes (blue) during cell division



Self-organization over 4 orders of magnitude

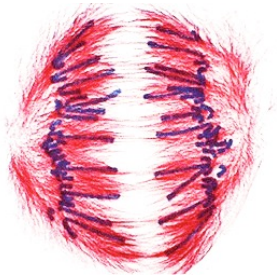


5 nm



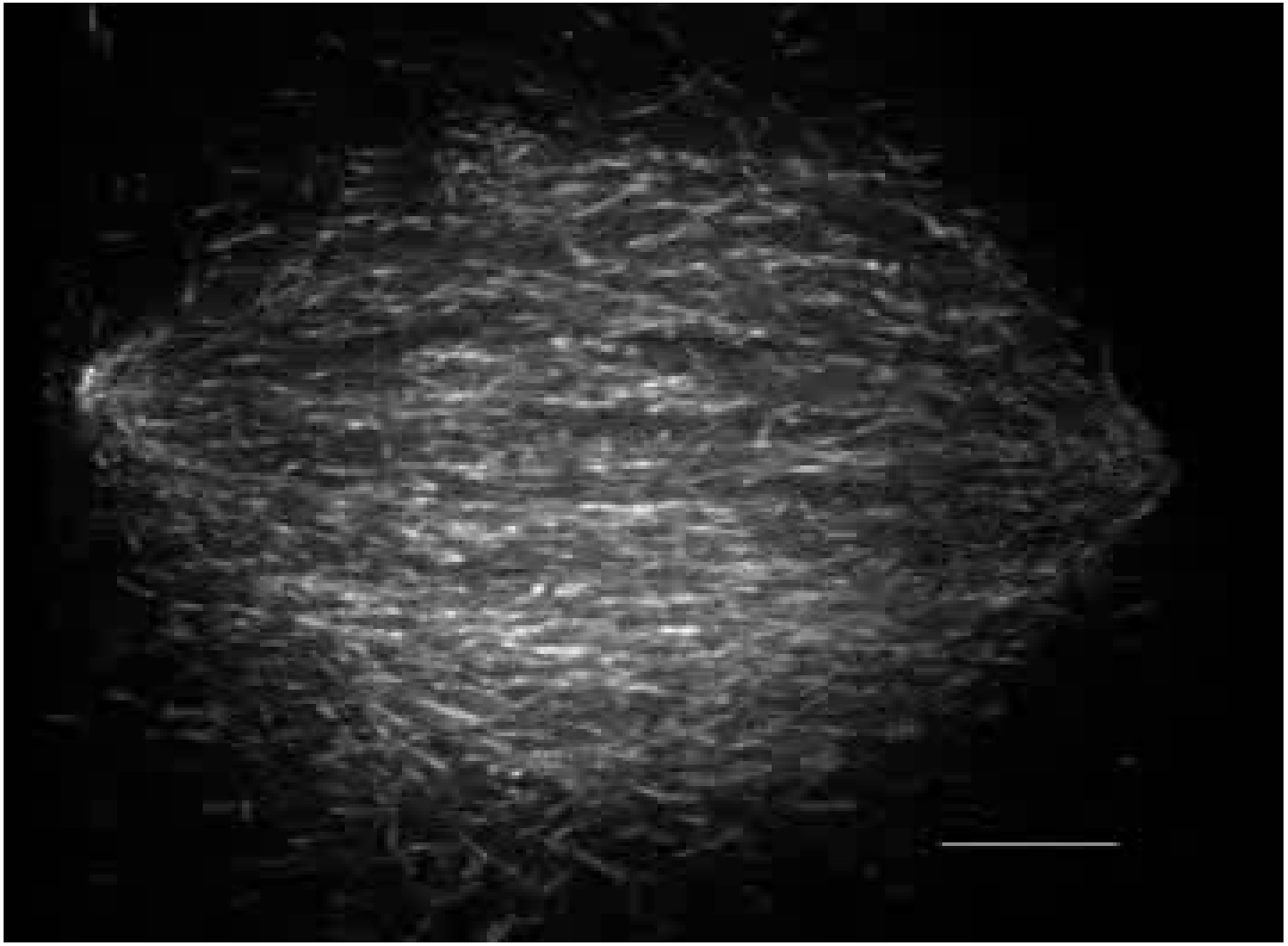
50 nm

0.5 µm



50 µm

The spindle

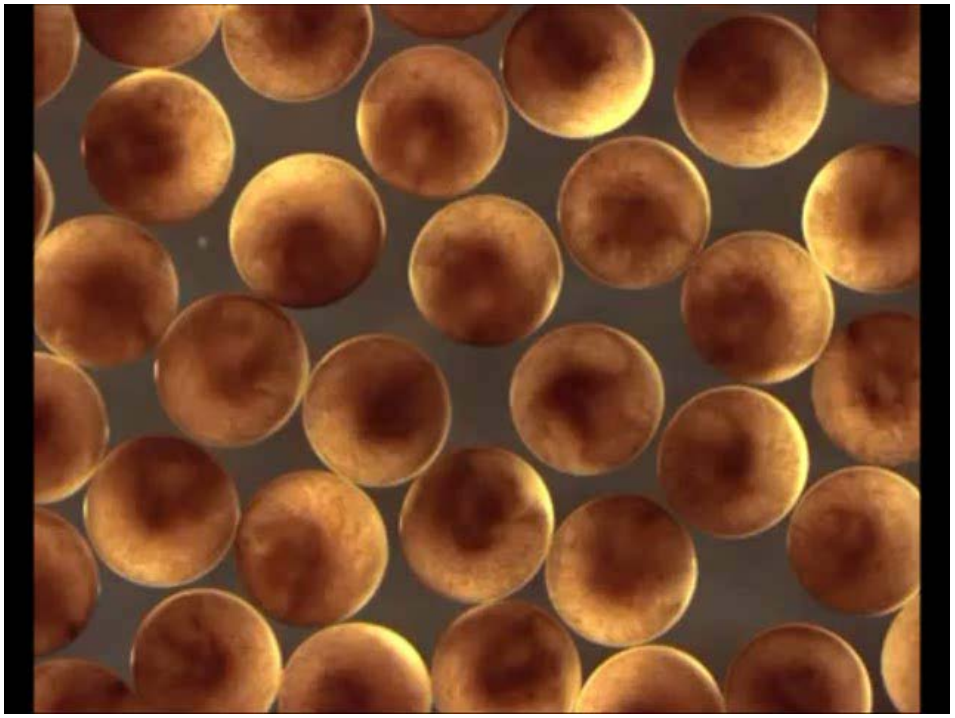


R. Laughlin and R. Heald

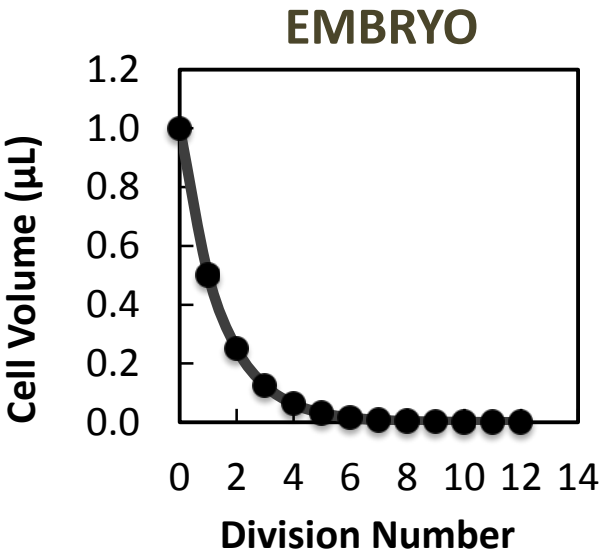
EB1-GFP

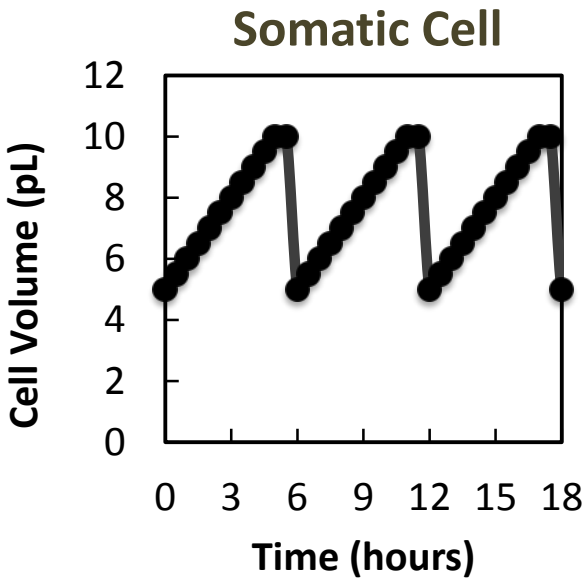
Cell size decreases during embryogenesis

SYSTEM = *Xenopus* Embryo Development



H. Williams and J. Smith

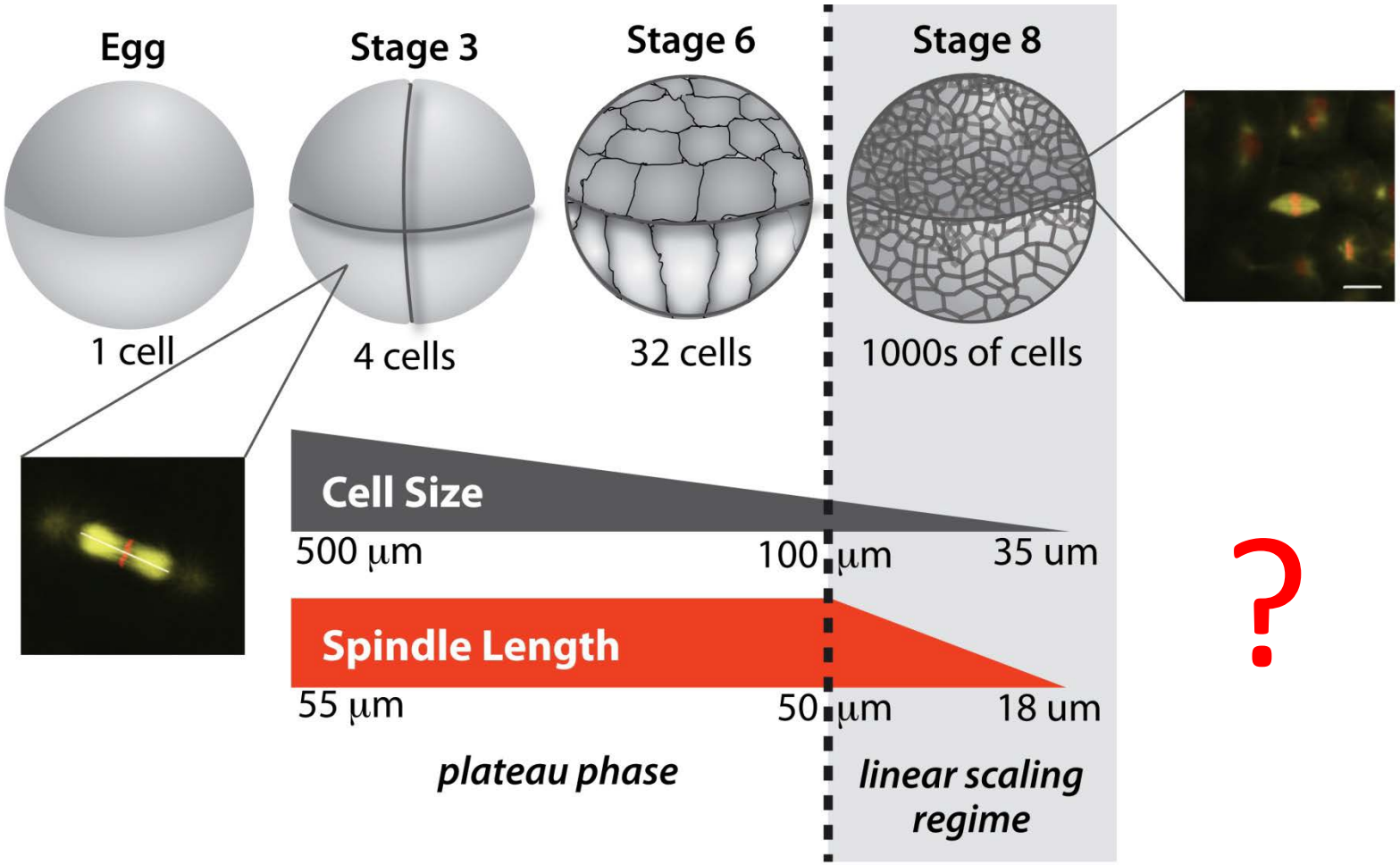




QUESTION: How do intracellular structures (organelles) adapt to the change in cell size?

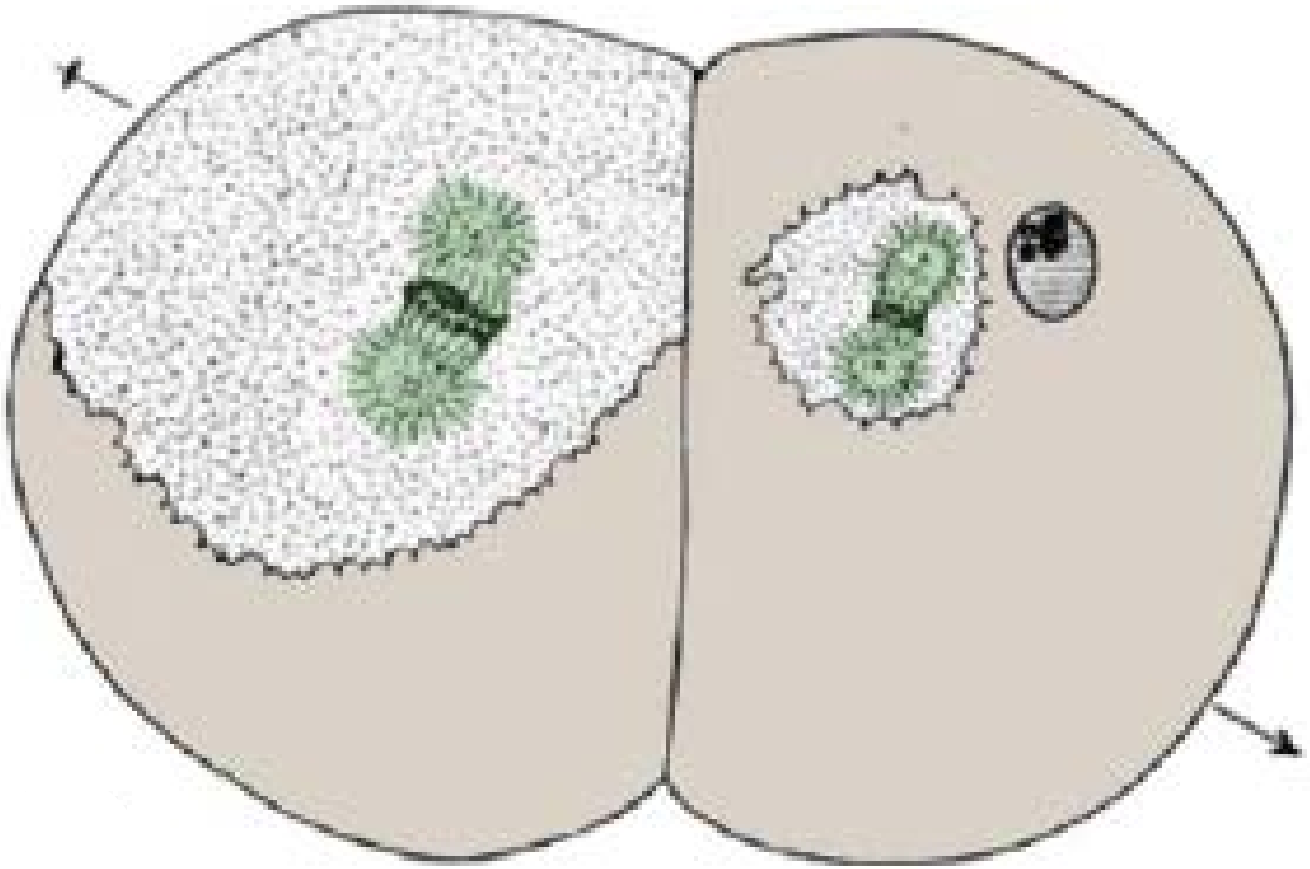
Matt Good, Mike Vahey, Rebecca Heald

Spindle size decreases during embryogenesis



Matt Good, Mike Vahey, Rebecca Heald

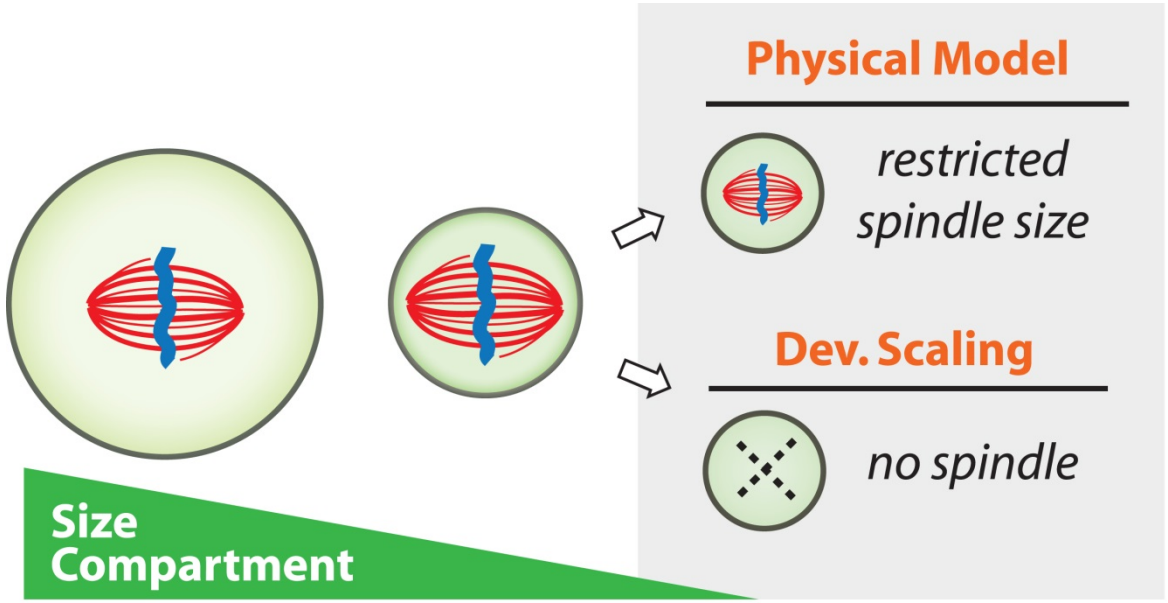
Spindle size scaling

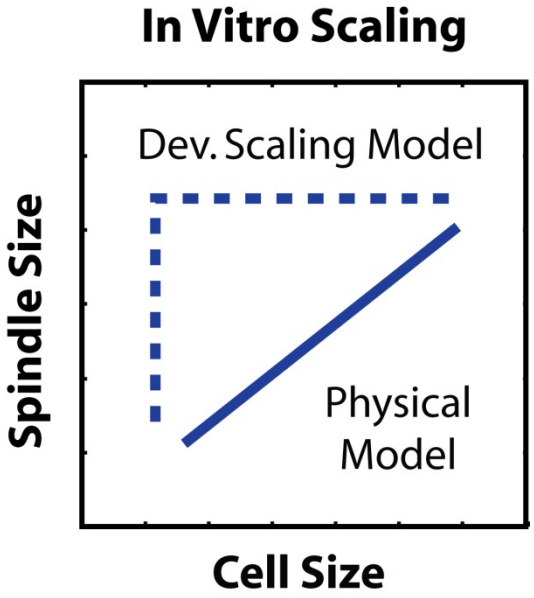


E. G. Conklin, 1912

Centrifuged crepidula (sea snail) 2-cell embryo

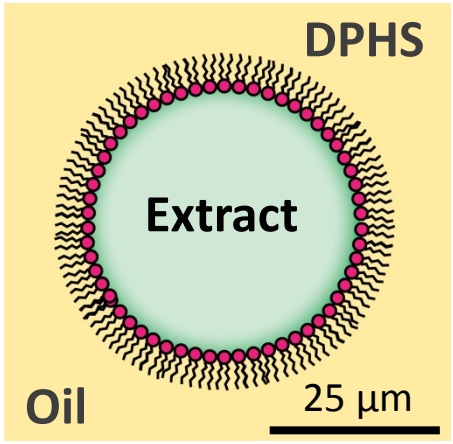
Cell & spindle regulation during development



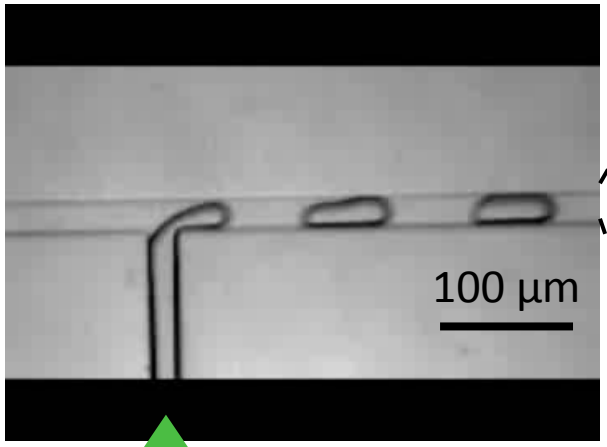


EXPERIMENAL TEST: Confine *Xenopus* egg extract spindles in cell-like compartments of defined size

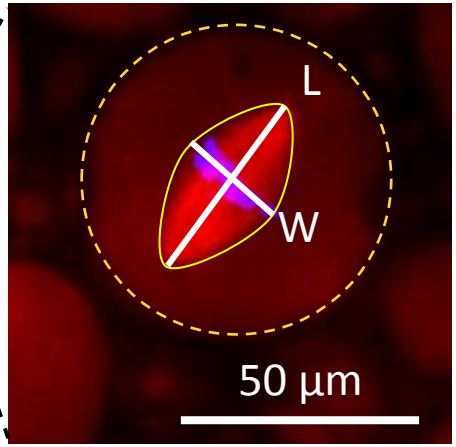
Encapsulation in cell-like compartments



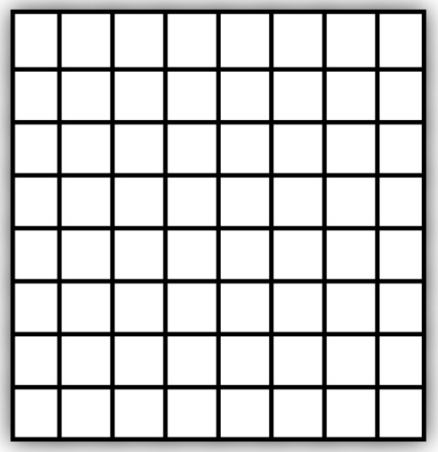
Oil +
Lipid



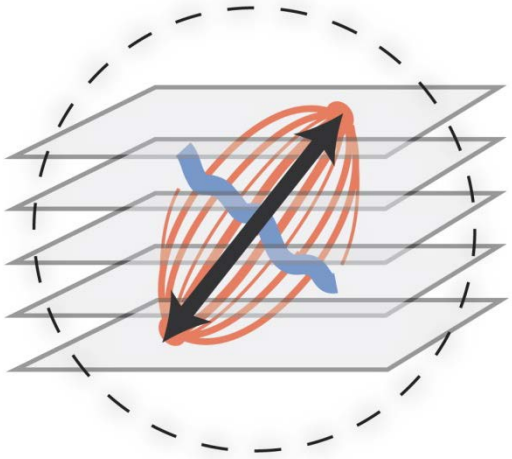
Extract + sperm



8 x 8 Scan / Stitch



Z-stacks

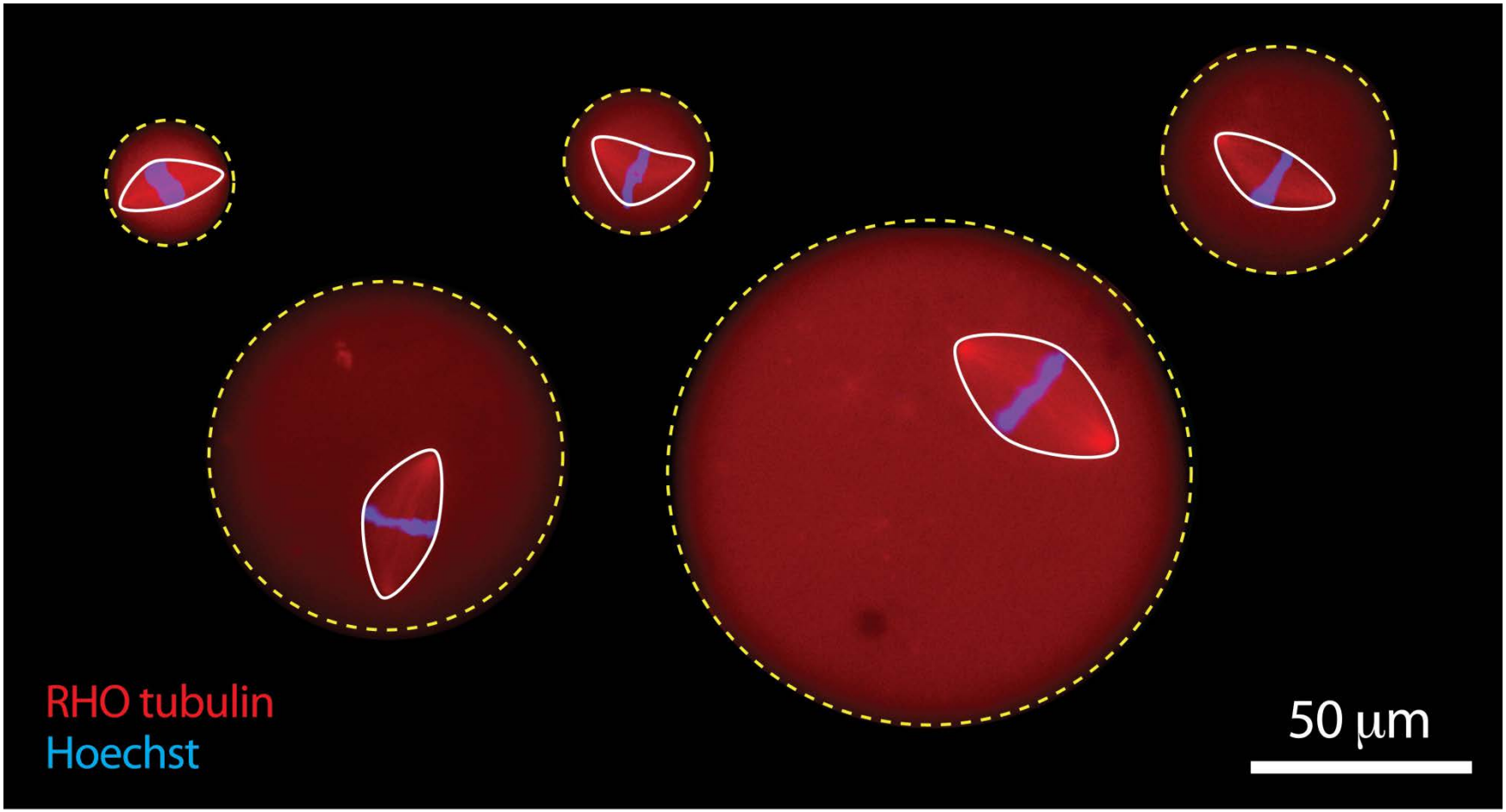


Measure

- Droplet Size
- Pole-to-pole L
- Spindle Width

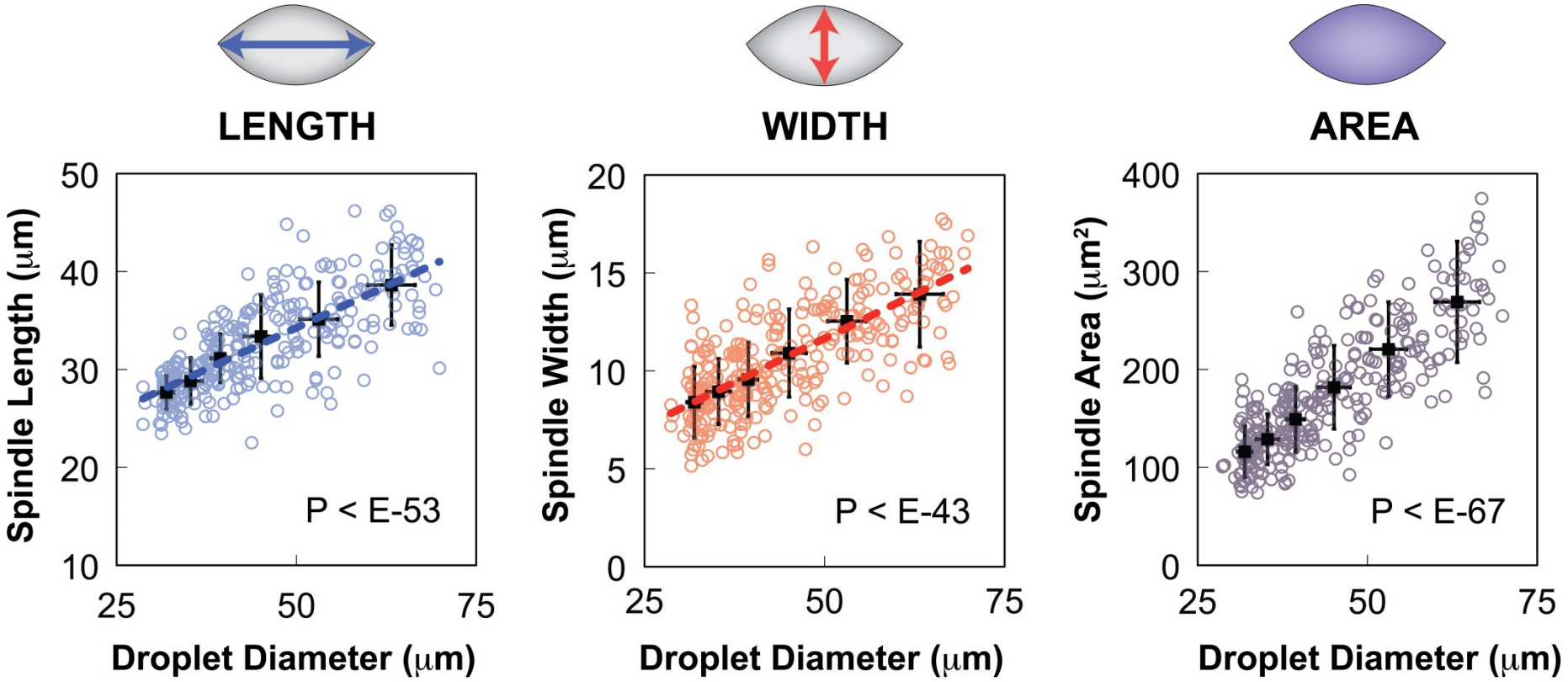
RHO Tubulin
DAPI DNA

Spindles in cell-like compartments



Matt Good, Mike Vahey, Rebecca Heald

Spindle size scales with compartment size



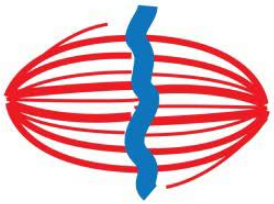
Extract unchanged!

Spindles simply assembled in compartments of varying size

Good, et al., Science, in press

How might volume affect assembly?

X. *laevis* Spindle
(Bulk Egg Extract)



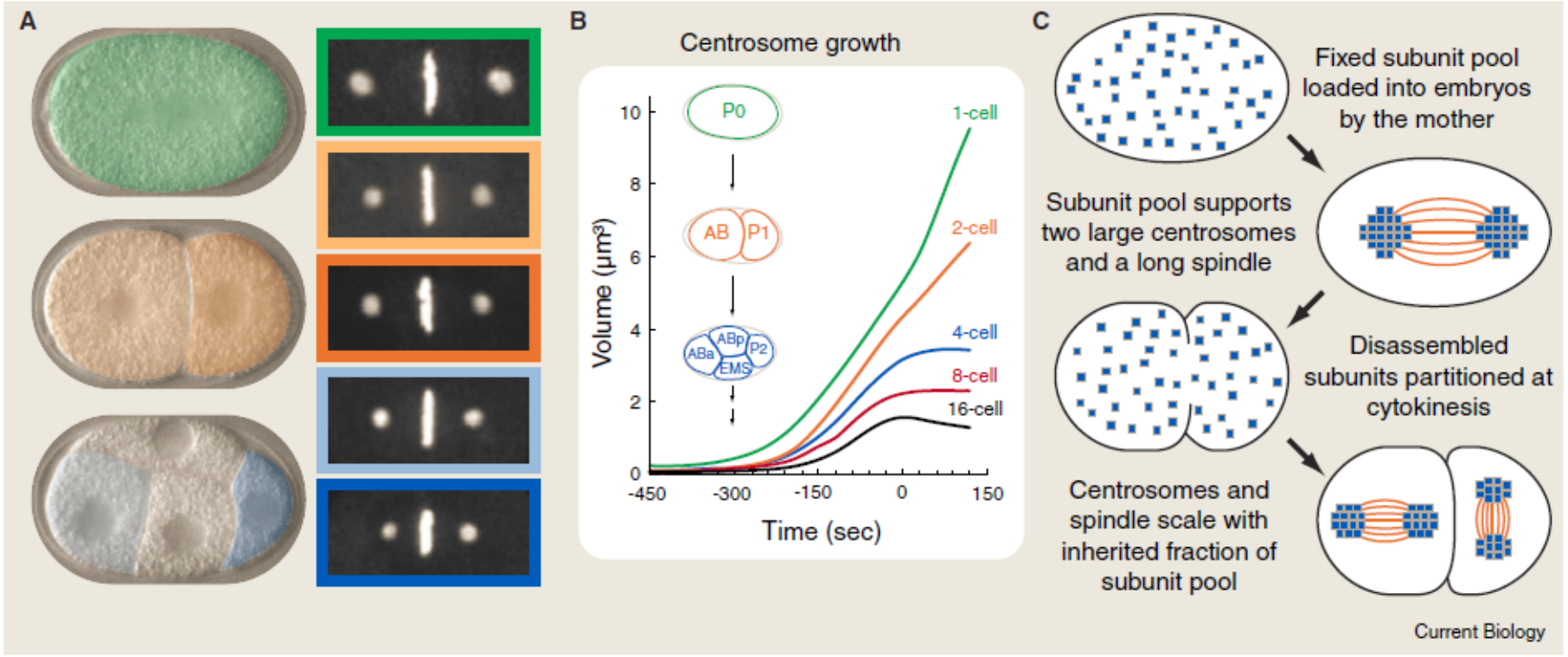
150,000 MTs
Avg Length = 7 μm

~ 3 x 10^9 required!

	Egg 1 cell	Stage 8 > 4000 cells
		
CELL DIAMETER	1000 μm	> 40 μm
# TUBULIN SUBUNITS	10^{13}	3×10^8
	$[Tubulin]_{cell} = 15\ \mu M$	**Insufficient** (Egg Spindle)

Reduced cell volume reduces # of spindle building blocks

Limiting components

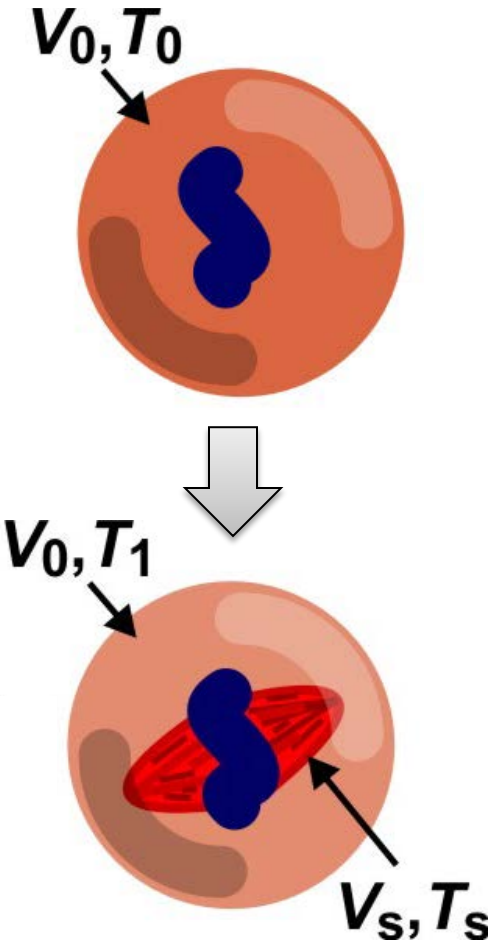


- Organelles form from a pool of components in the cytoplasm
- Growth rates often depend on concentration of components
- As cell volume changes, the number of components may become limiting, even if concentration does not change

Goehring & Hyman, Current Bio, 2012

Lesson 1: Volume can control scaling

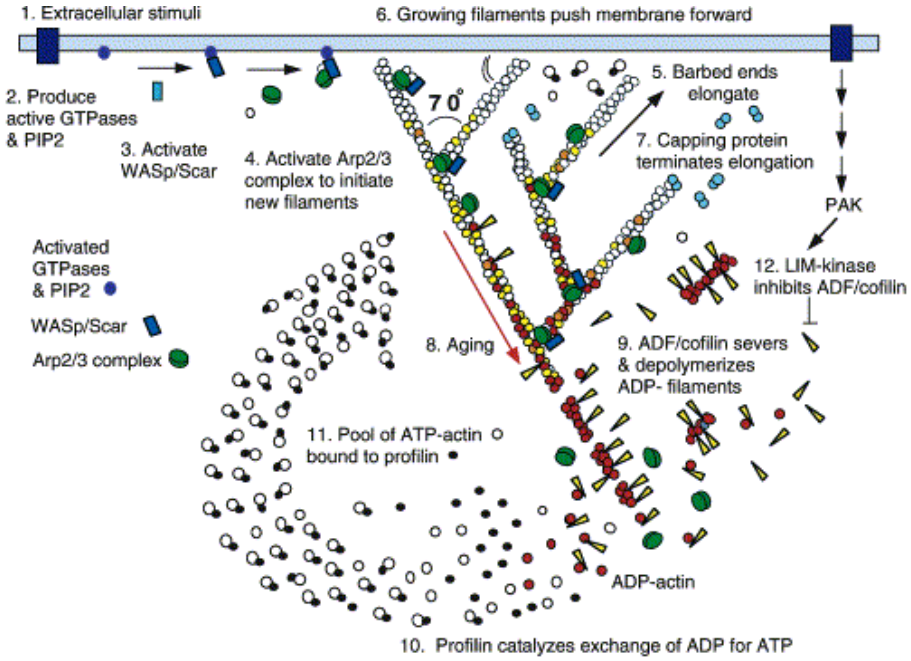
- Conservation of tubulin forms the basis of a simple model of spindle scaling.
- The model predicts two regimes (linear & plateau) that are observed experimentally.
- The model is numerically consistent with experimental data over a broad range of cell/droplet sizes.
- Tubulin is necessary but not sufficient; other components are likely also limiting.
- The model can be generalized to account for non-linear dependencies on tubulin (or other protein) concentrations, diffusion, etc.



Matt Good, Mike Vahey, Rebecca Heald

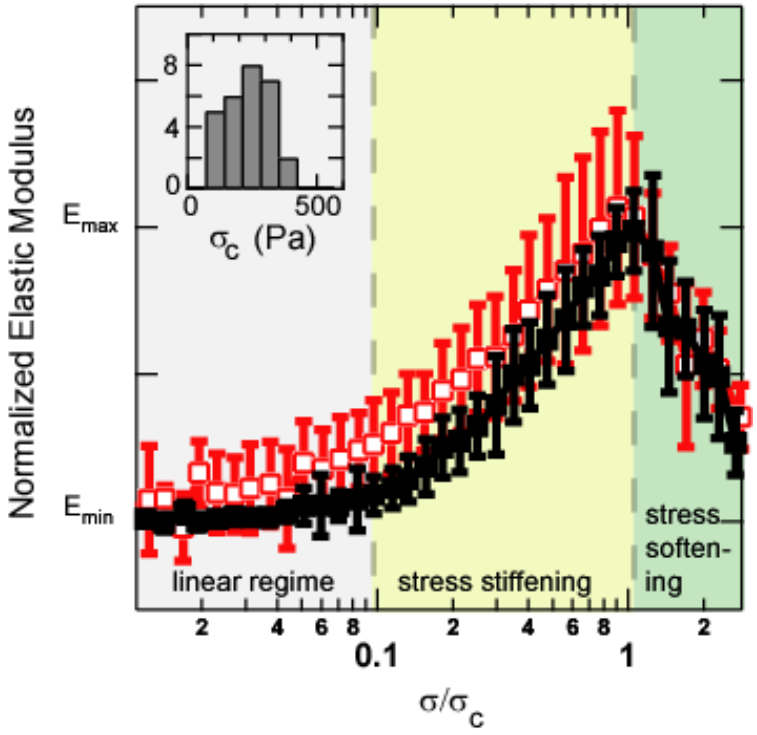
Lesson 2: Architecture controls elasticity

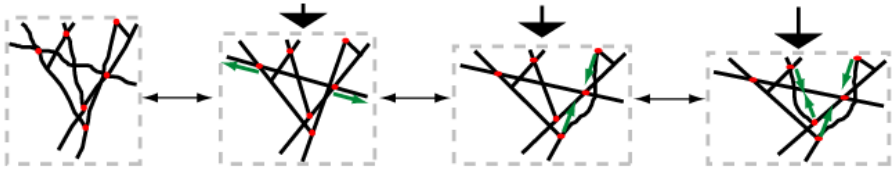
Nucleation and polymerization of actin at the leading edge generates protrusive forces



Pollard and Borisy, *Cell,* 2003

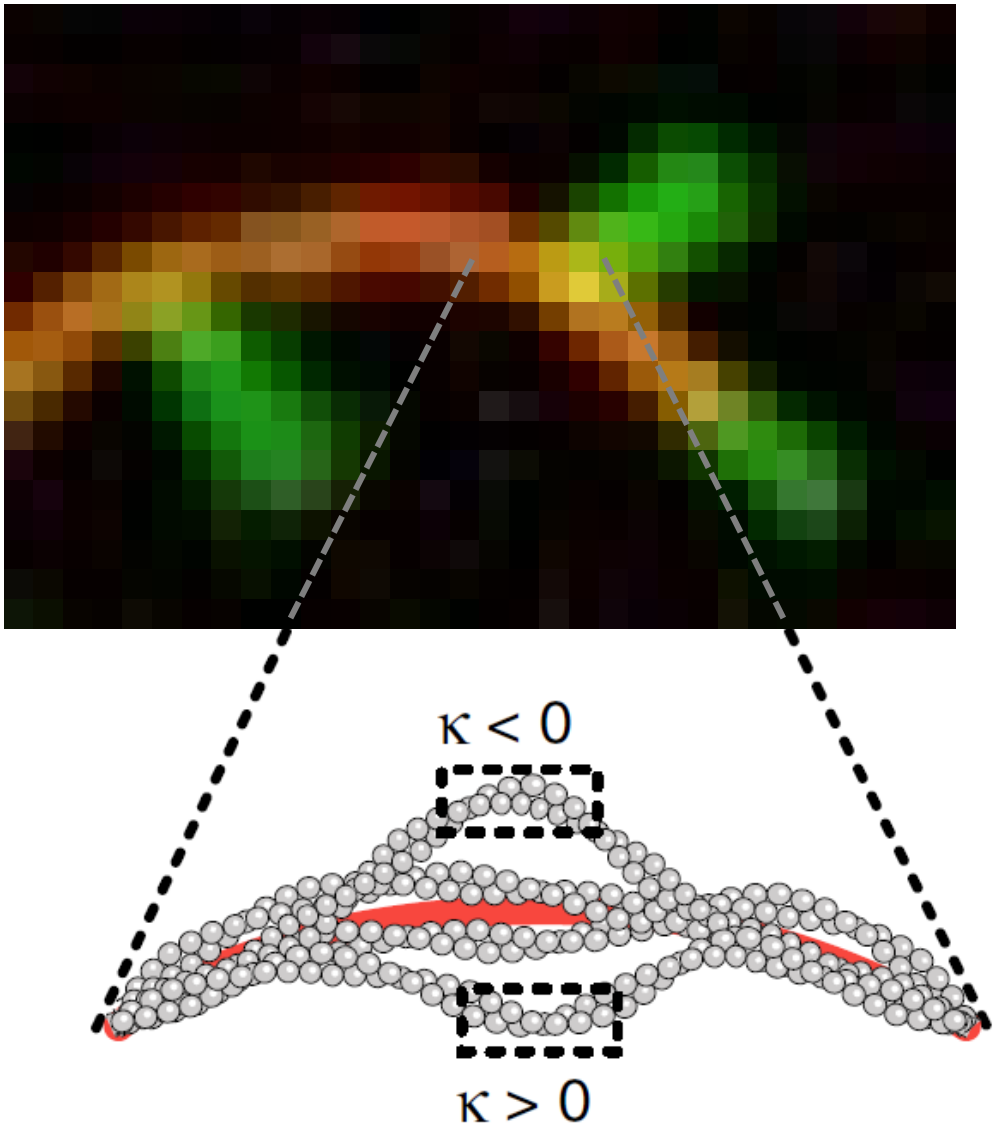
Networks reversibly stiffen and soften as load increases.



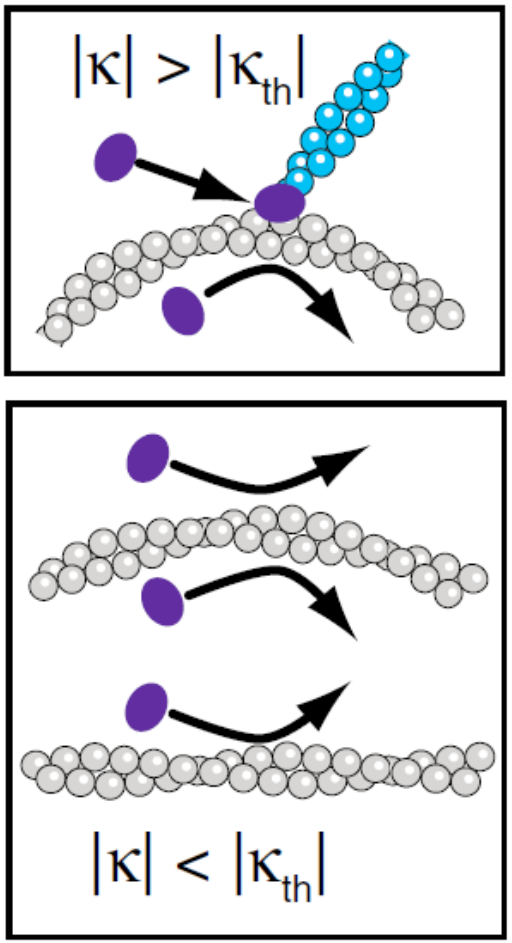


Chaudhuri et al., Nature, 2007

Lesson 3: Bending alters binding

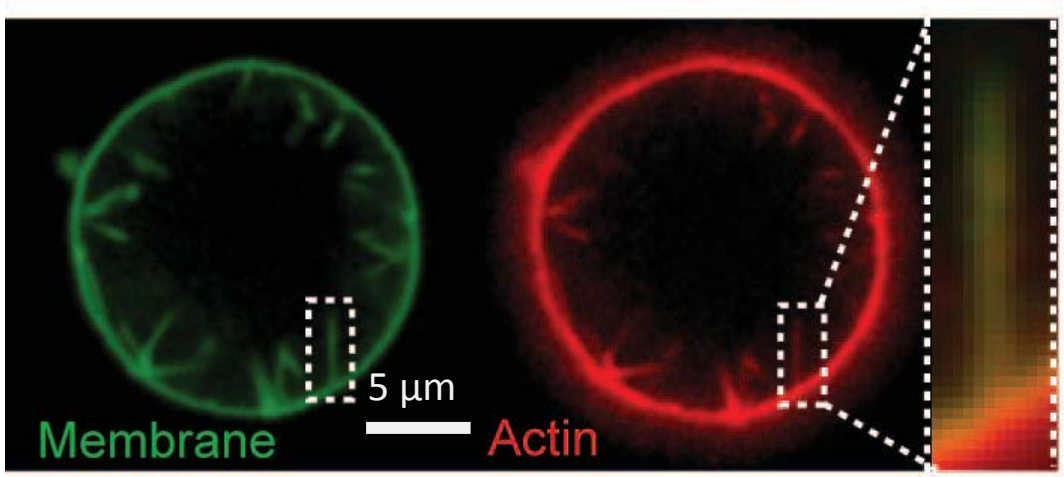


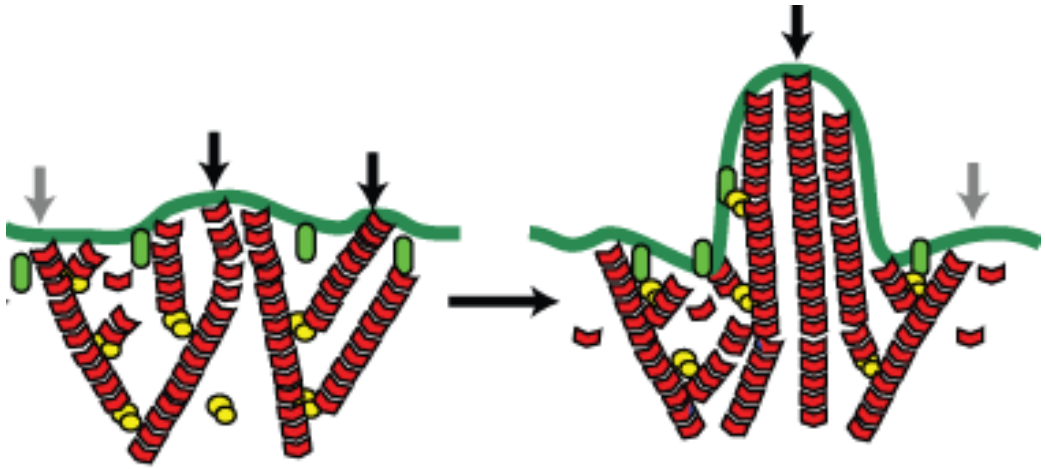
Arp2/3 favors the outside of a bent filament

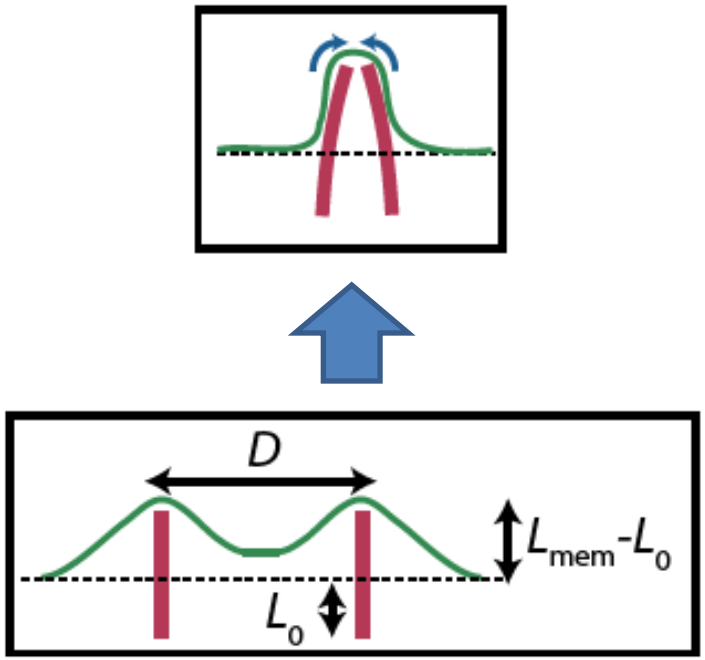


Risca et al., PNAS, 2012

Lesson 4: Membranes bundle & stabilize

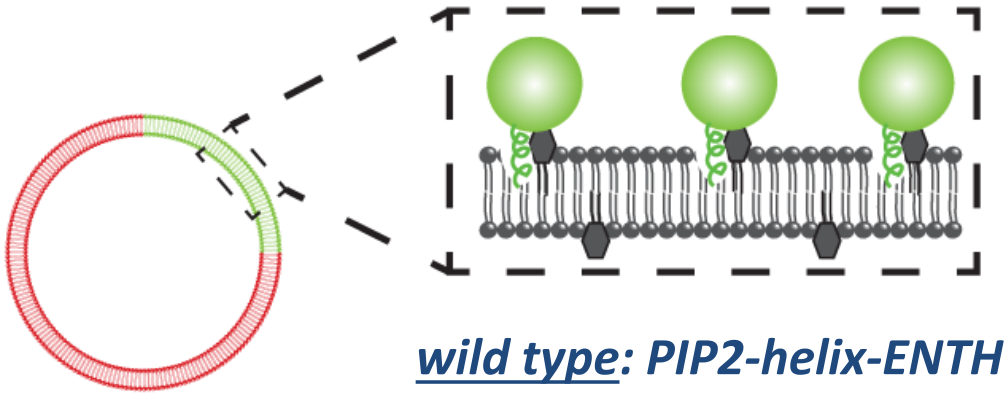




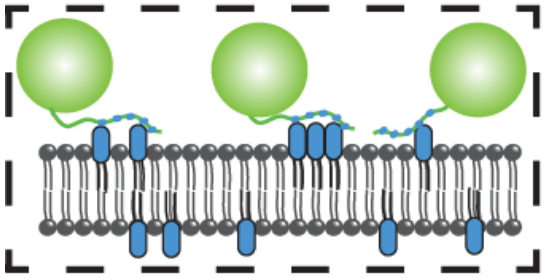


Liu et al., Nature Physics, 2007

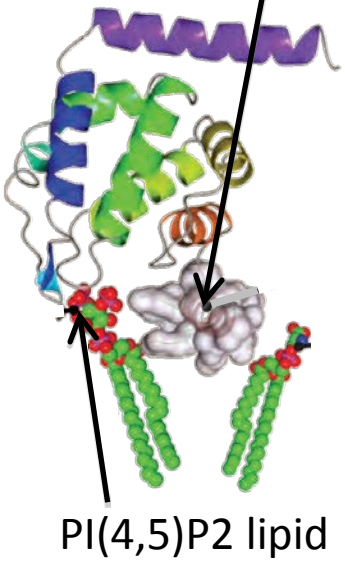
Lesson 5: Crowding induces curvature

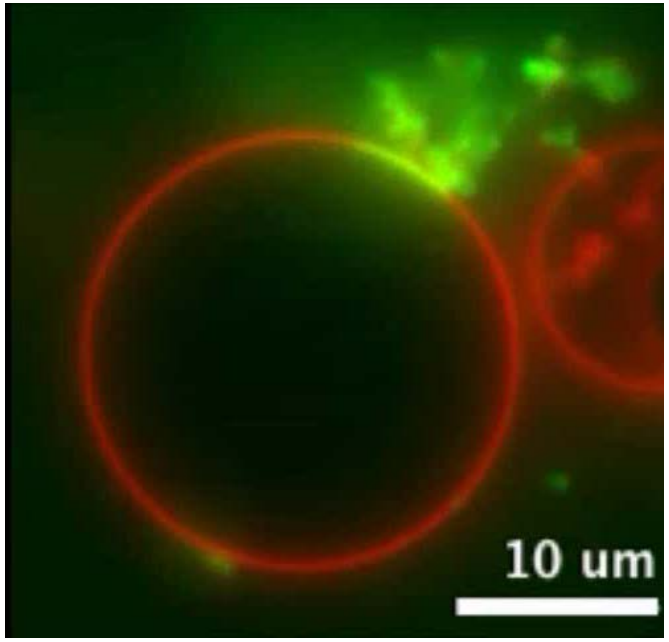


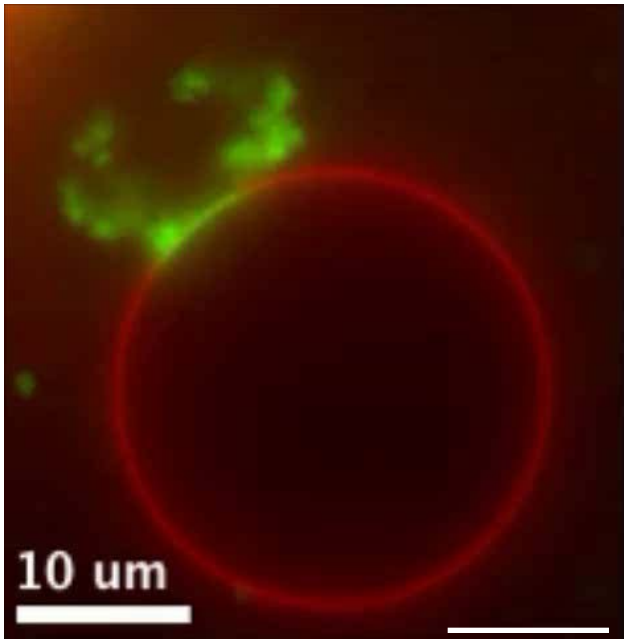
***wild type*: PIP2-helix-ENTH**



***mutant*: NiNTA-his6-ENTH**





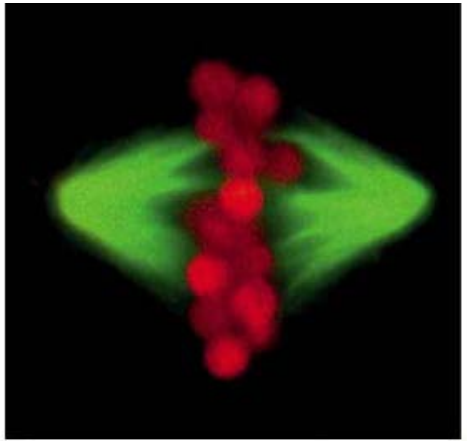


Stachowiak, Schmid, et al., Nature Cell Biology, 2013

Toward self-organized biological materials

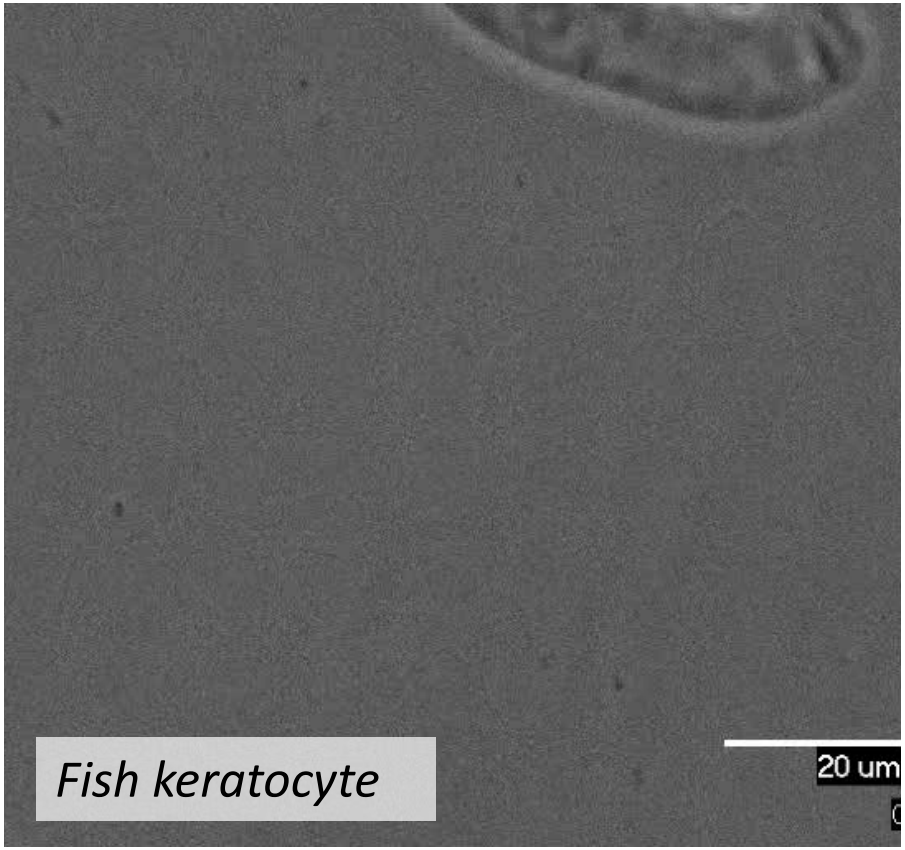
Ingredients (molecules) + instructions (constraints) are necessary to reconstitute biological structures.

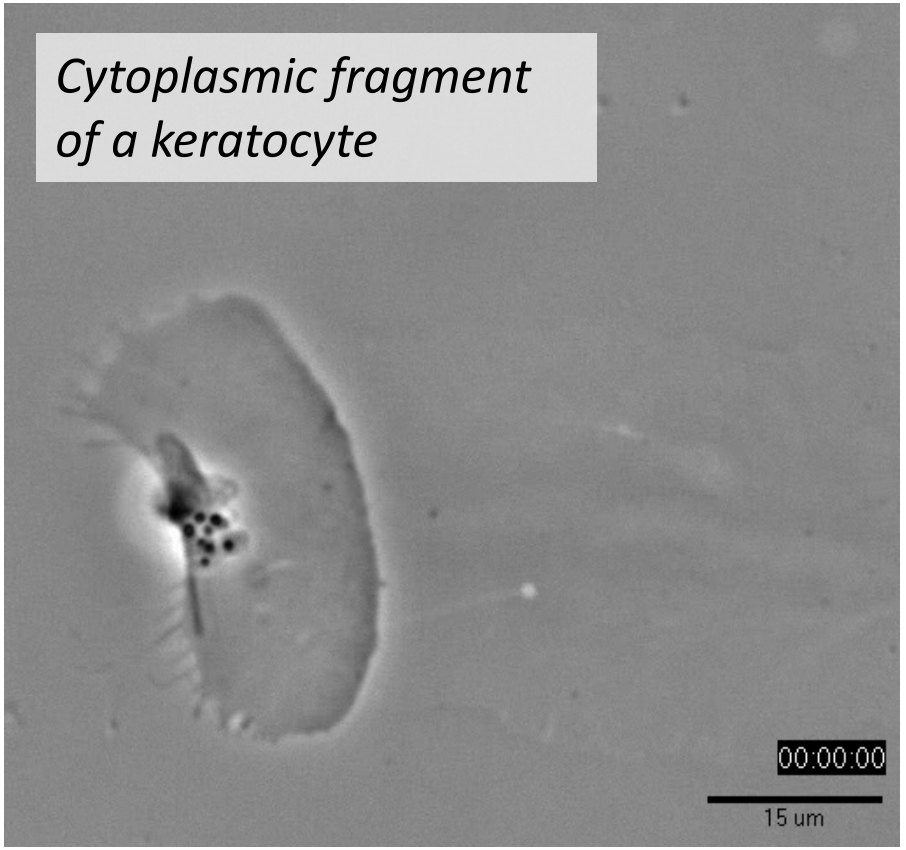
- Lesson 1: Volume can control scaling
- Lesson 2: Architecture controls elasticity
- Lesson 3: Bending alters binding
- Lesson 4: Membranes bundle & stabilize
- Lesson 5: Crowding induces curvature
- Etc, etc, etc.



How can we form more complex biological structures?
How do we confirm biological function?

Alive or dead?





Do we need a Turing test for biological materials?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Rebecca Heald (UC Berkeley)
Dyche Mullins (UCSF)
Carl Hayden (Sandia)
Darryl Sasaki (Sandia)