Muscle Organization and Dynamics and its Role in Morphogenesis in Hydra Regeneration

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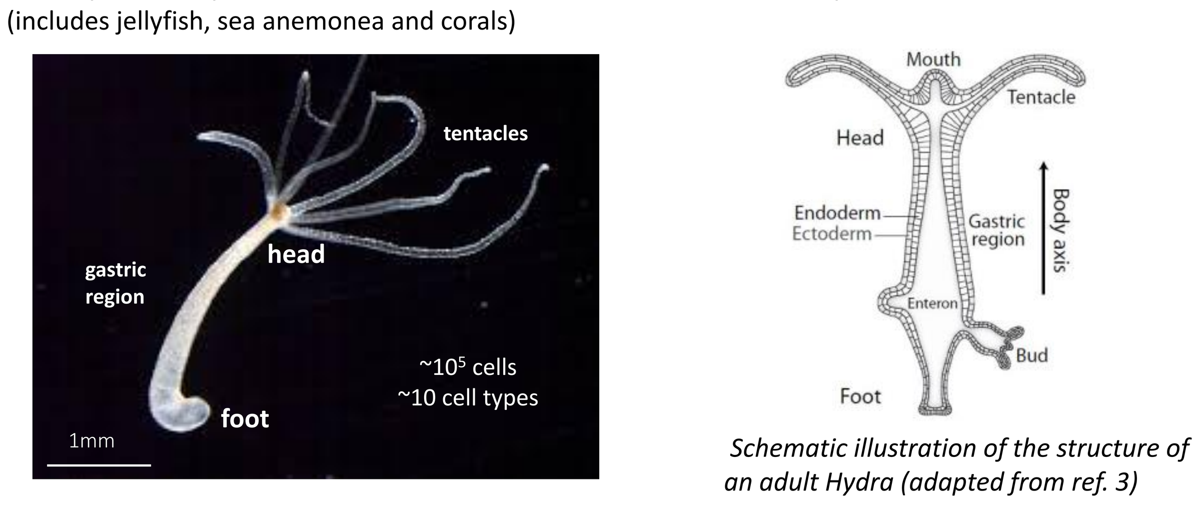
Animal morphogenesis arises from the complex interplay between multiple mechanical and biochemical processes with mutual feedback. Developing an effective, coarse-grained description of morphogenesis is essential for understanding how these processes are coordinated across scales to form robust, functional outcomes. Here we show that the nematic orientation field of the supra-cellular muscle fibers in regenerating *Hydra* provides an effective description of the morphogenesis process. We show that topological defects in this field act as organization centers with morphological features developing at defect sites. Furthermore, we investigate the role of muscle organization, the forces they exert, and how these affect cell and tissue dynamics, with the purpose of understanding the mechanism driving body axis elongation and the formation of morphological features at defect sites.

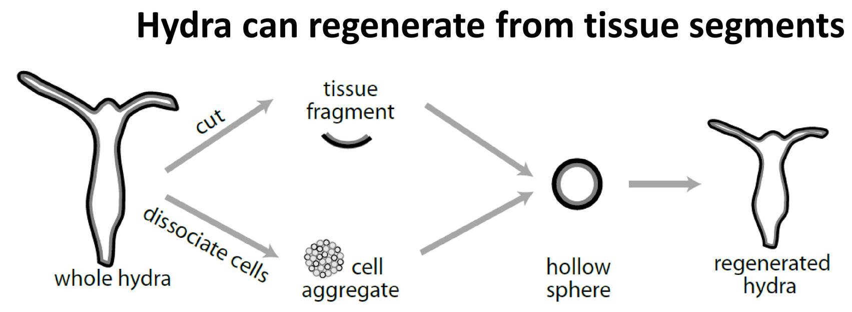
Hydra structure and regeneration

Small predatory fresh water animal from the Cnidarian family (includes jellyfish, sea anemonea and corals)



Schematic illustration of the structure of an adult Hydra (adapted from ref. 3)

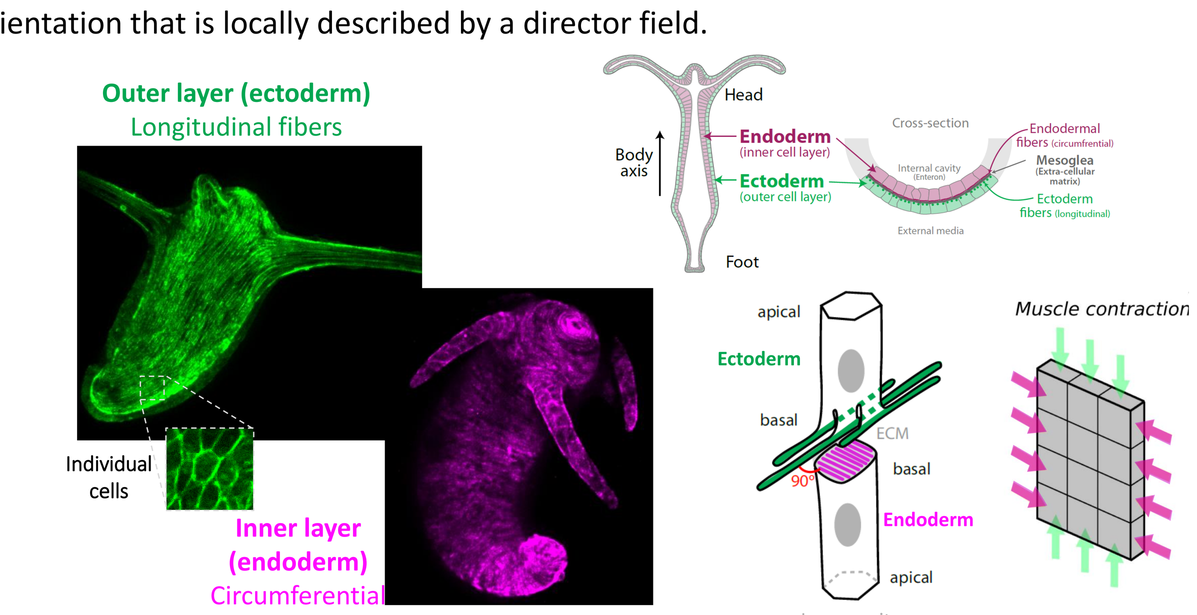
Hydra can regenerate from tissue segments or cell aggregates



- Requires a minimal size (10^2s-10^4 cells).
- Does not require cell proliferation
- Occurs on time scales of 24-72 hours.

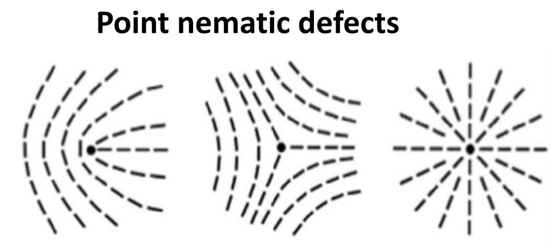
Nematic organization of Hydra actin fibres

Nematic organization: actin fibres align parallel to each other, along a preferred orientation that is locally described by a director field.



Singularities in nematic organization

Point nematic defects



Topological charge: number of times the director rotates along a path encircling the defect - $S = \frac{1}{2\pi}\oint d\theta$.

A nematic on a spheroid must have a total charge of +2.

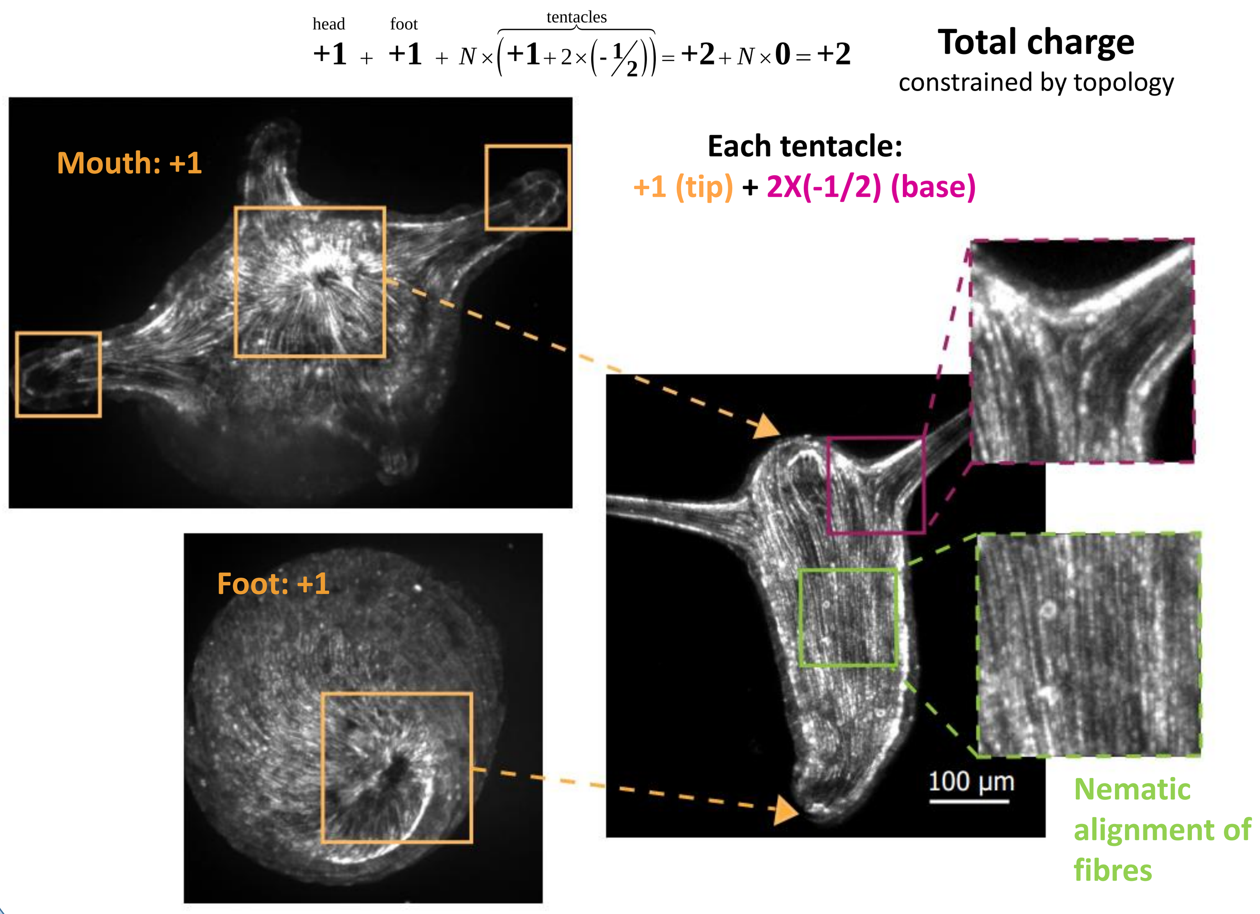
Nematic organization in mature Hydra

Defects located at **morphological features**, at regions of **highest curvature**

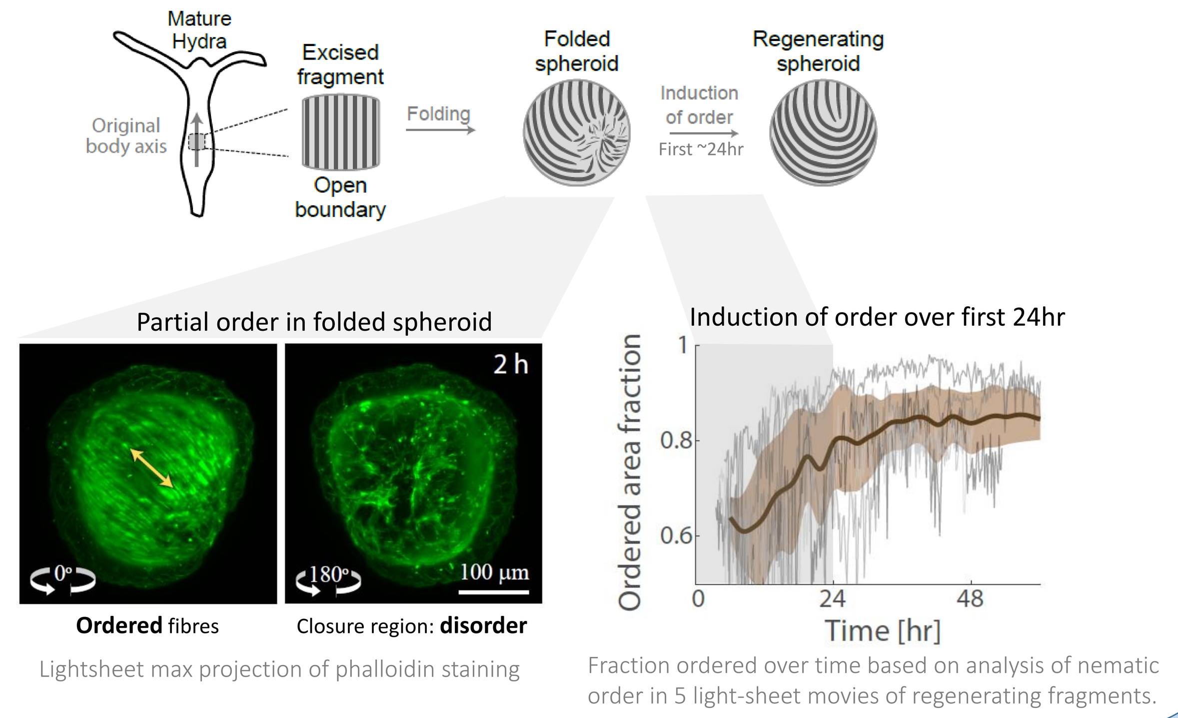
$$\underset{head}{+1} + \underset{foot}{+1} + N\times\underset{tentacles}{\left(+1+2\times\left(-\tfrac{1}{2}\right)\right)} = +2 + N\times 0 = +2$$

Total charge constrained by topology

Each tentacle: +1 (tip) + 2X(-1/2) (base)



Inheritance of nematic alignment and induction of order from ordered domain

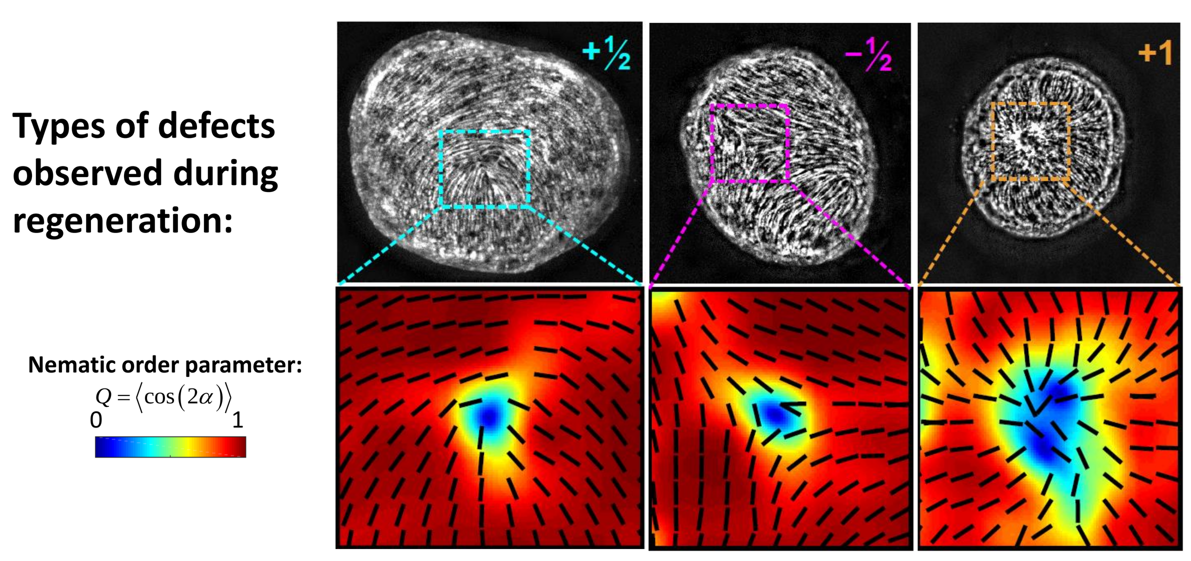


Lightsheet max projection of phalloidin staining

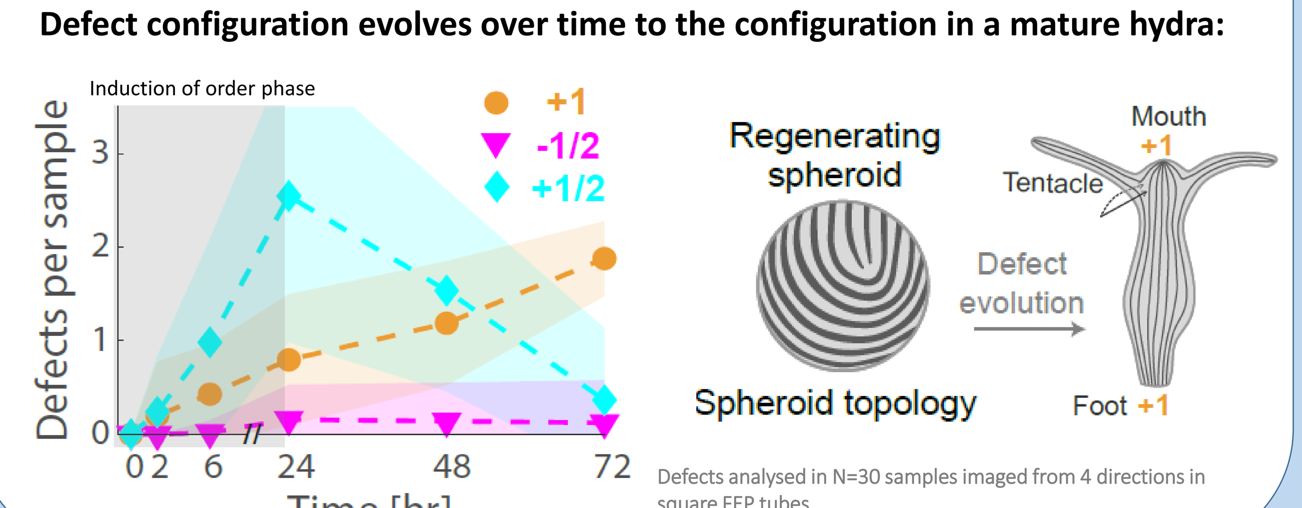
Fraction ordered over time based on analysis of nematic order in 5 light-sheet movies of regenerating fragments.

Evolution of nematic defect configuration during Hydra regeneration

Types of defects observed during regeneration:



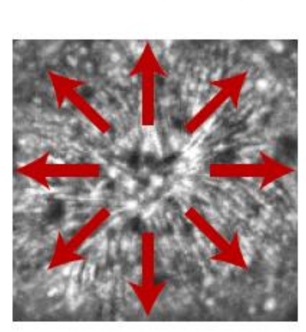
Defect configuration evolves over time to the configuration in a mature hydra:



Defects analysed in N=30 samples imaged from 4 directions in square FEP tubes

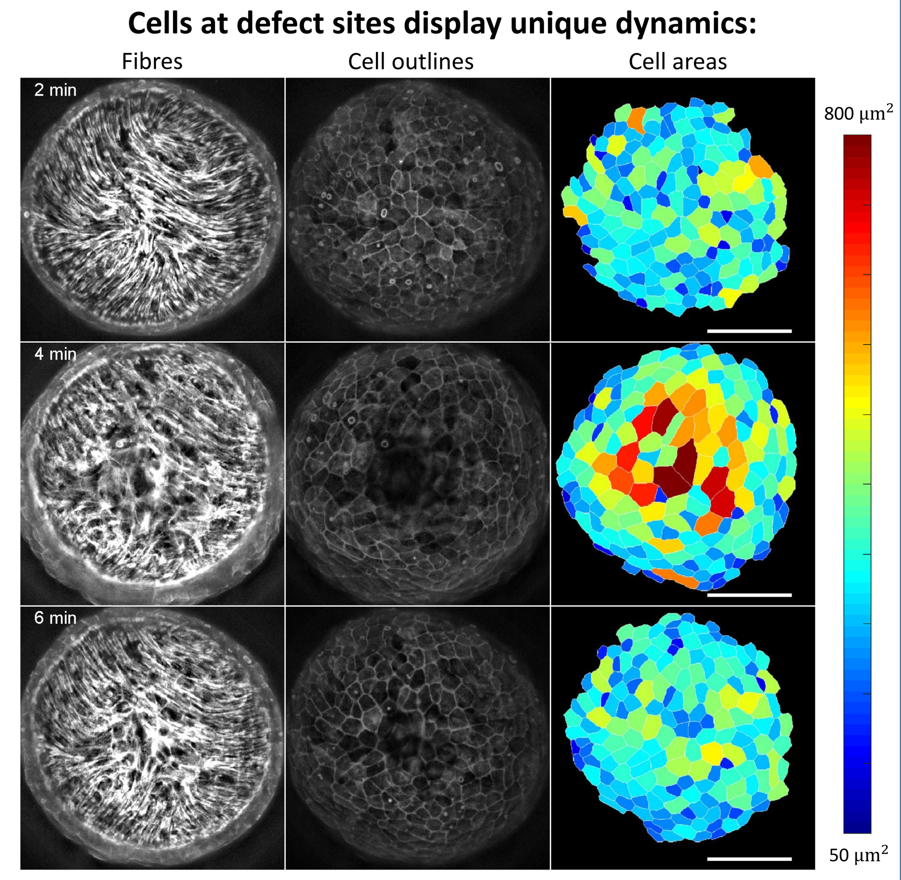
Defect sites are characterized by a unique mechanical environment

Defect sites are structurally unique:

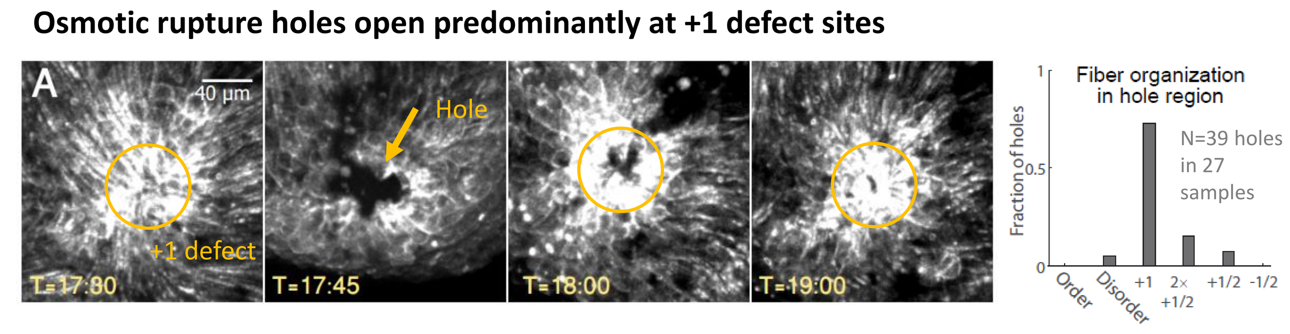


→ Weaker adhesion
→ Higher tension

Cells at defect sites display unique dynamics:

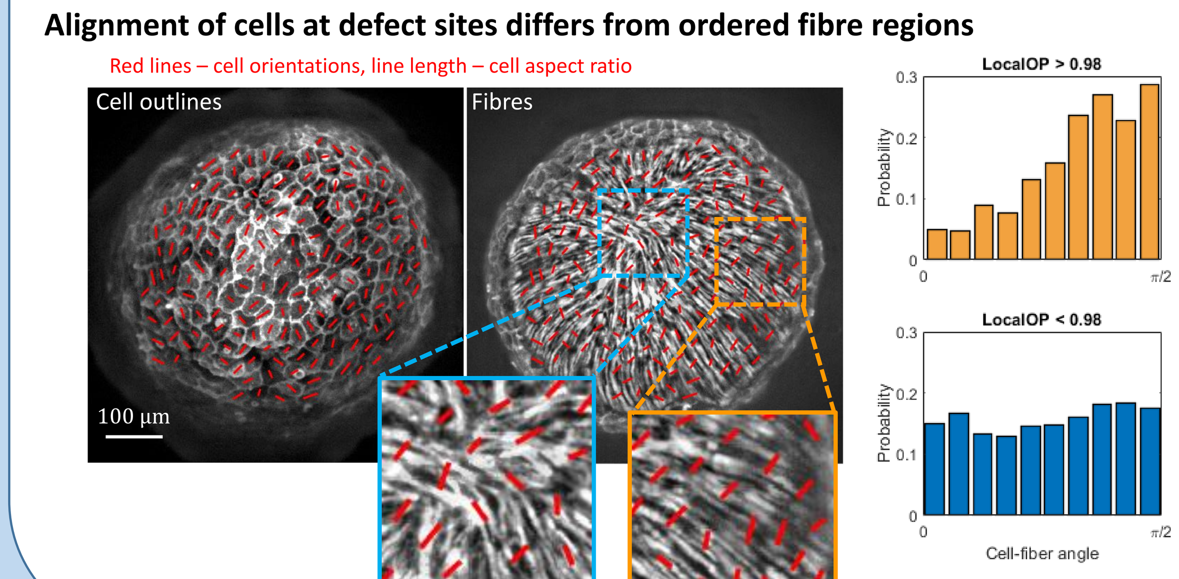


Osmotic rupture holes open predominantly at +1 defect sites



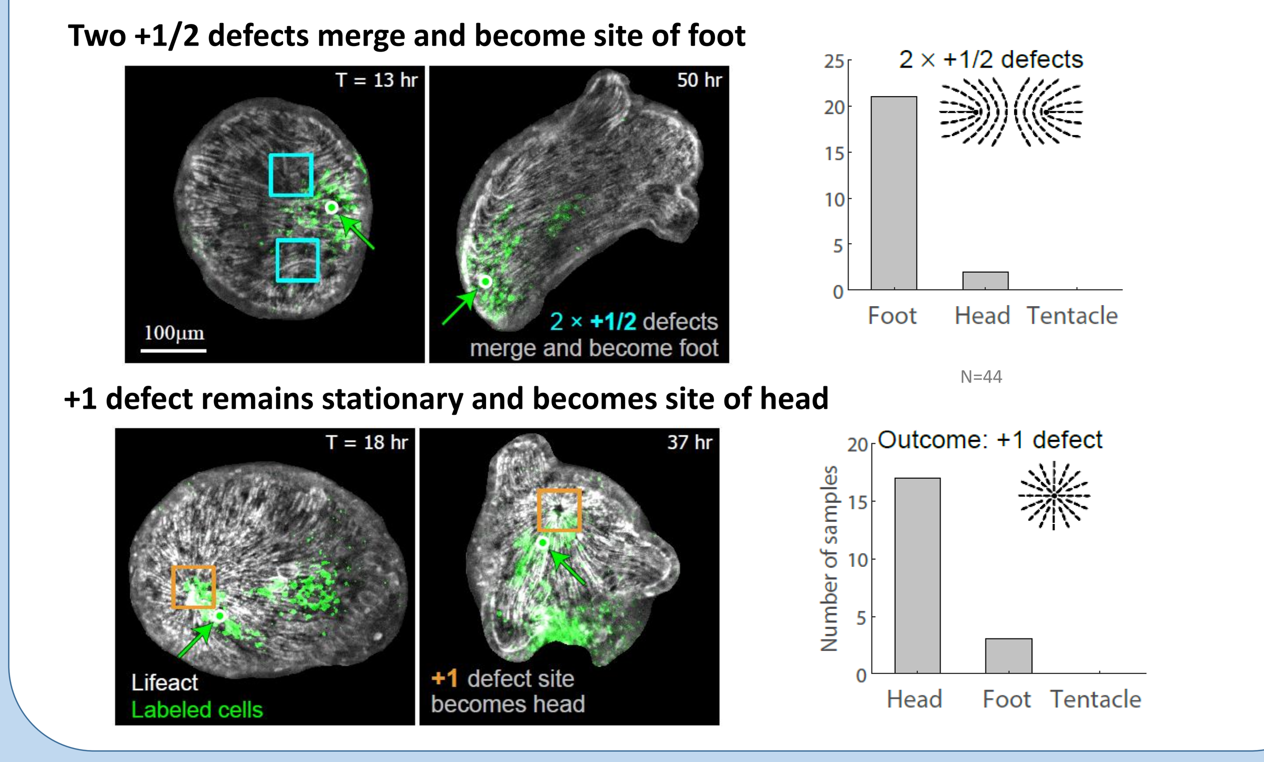
Alignment of cells at defect sites differs from ordered fibre regions

Red lines – cell orientations, line length – cell aspect ratio



N=2776 cells from 30 samples, cell aspect ratio >1.25

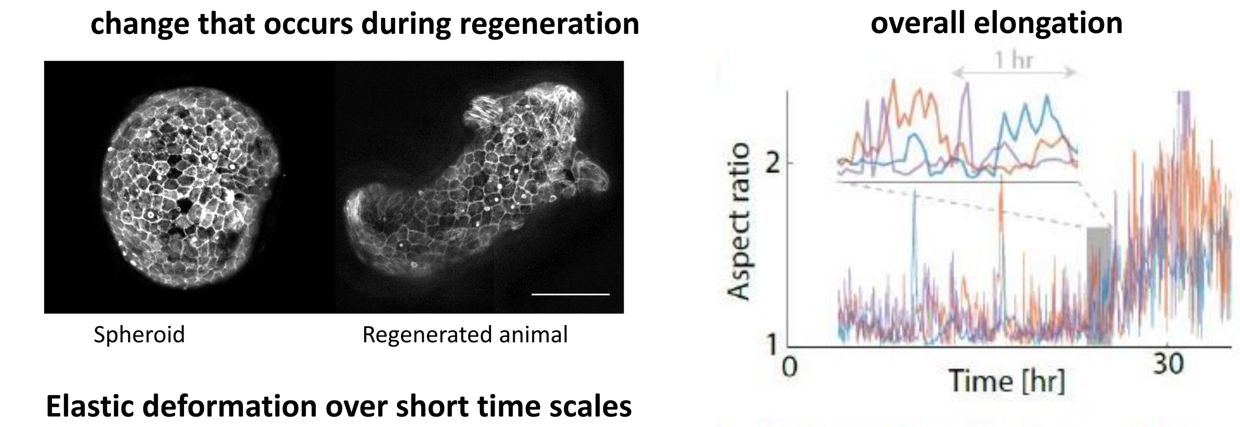
The formation of the head and foot is directly related to defect dynamics



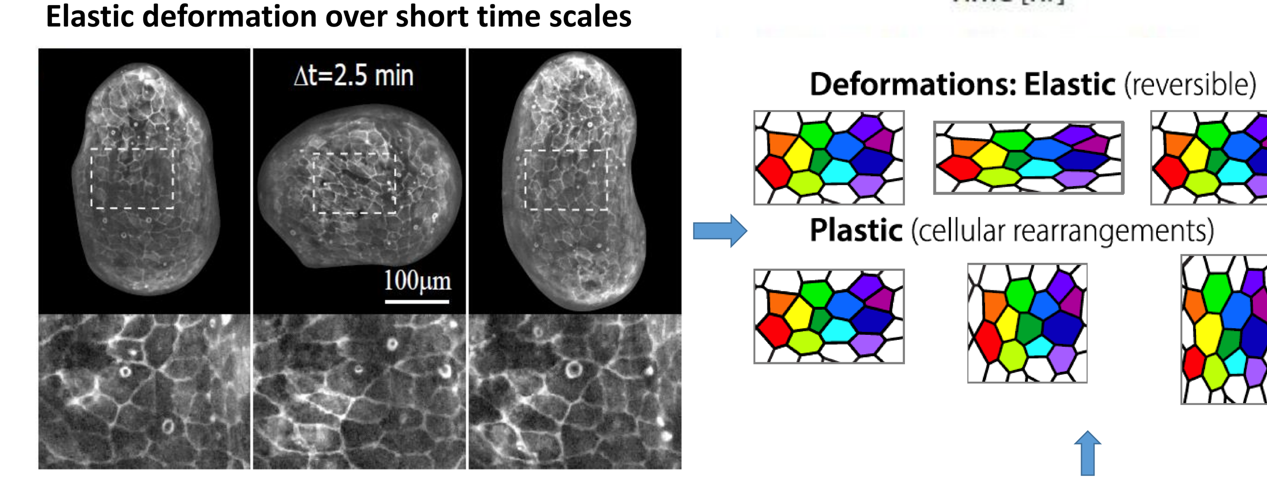
What drives body axis elongation during regeneration?

Body axis elongation is the major morphological change that occurs during regeneration

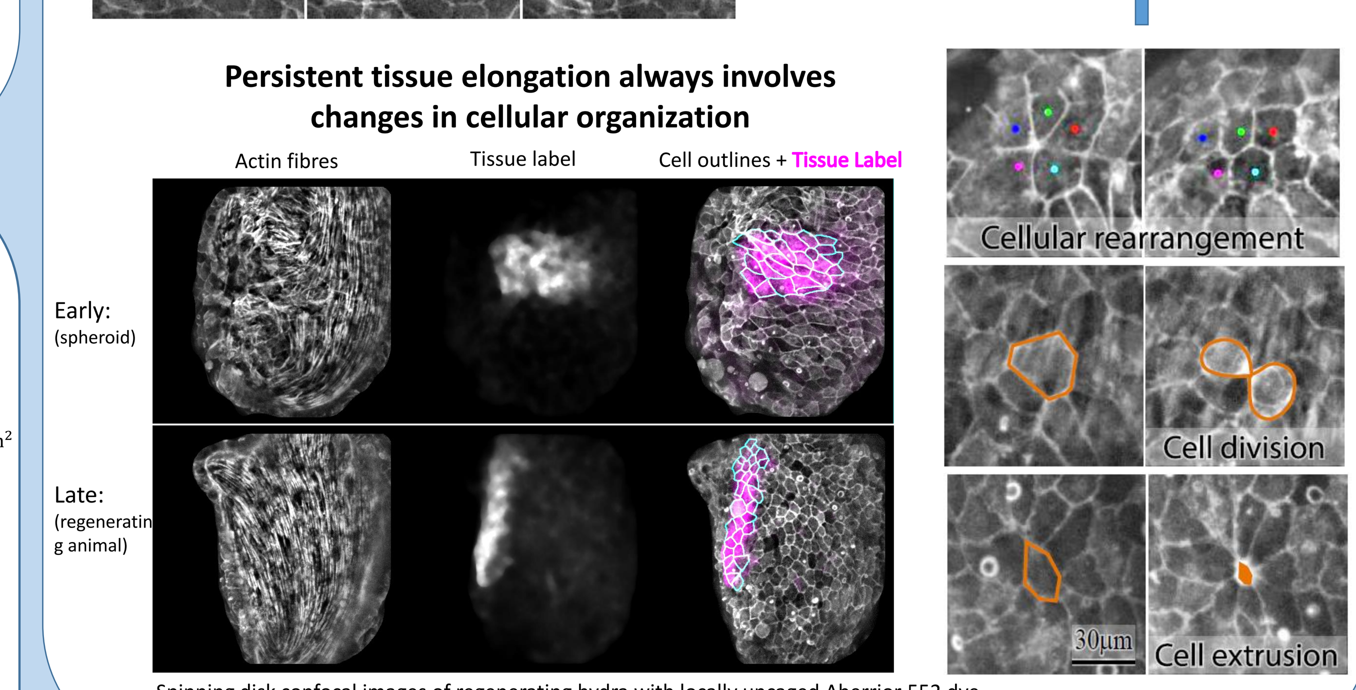
Fluctuations are comparable to overall elongation



Elastic deformation over short time scales



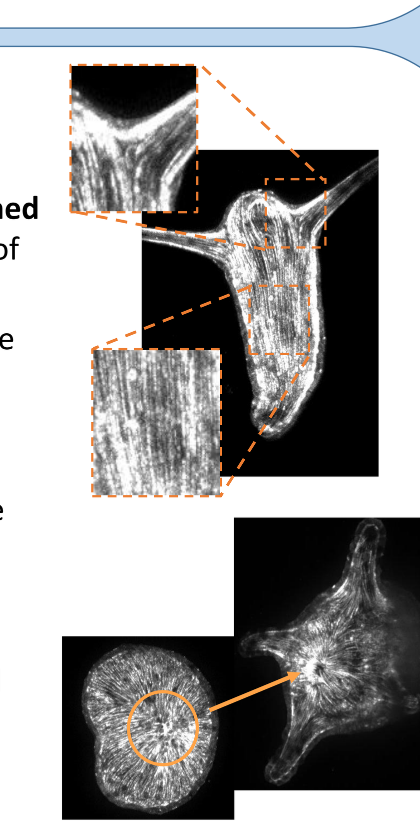
Persistent tissue elongation always involves changes in cellular organization



Spinning disk confocal images of regenerating hydra with locally uncaged Aberrior 552 dye

Summary

- The **actin fiber orientation field** provides a **coarse-grained field** whose dynamics provide an **effective description** of ***Hydra* morphogenesis.**
- **+1 defects** can be identified early, remain stable, and are characterized by a **unique mechanical environment**, providing the location of **organization centres** for the formation of **morphological features**.
- Regeneration involves large, fluctuations in tissue shape and forces, ultimately resulting in persistent **body axis elongation** that involves cell rearrangement.
- These suggest a process of self-organisation involving **mechanical feedback** between muscle organization and activity, tissue dynamics, and the resulting stress fields.



Nematic orientation field as a **"mechanical morphogen"** that can interact with other **mechanical** and **biochemical morphogens, leading** to the robust formation of the body plan in regenerating *Hydra.*

References:

(1) Maroudas-Sacks, Y., Garion, L., Shani-Zerbib, L., Livshits, A., Braun, E., & Keren, K. (2021). Topological defects in the nematic order of actin fibres as organization centres of *Hydra* morphogenesis. *Nat. Phys.* 17, 251–259.
(2) Livshits, A., Shani-Zerbib, L., Maroudas-Sacks, Y., Braun, E., & Keren, K. (2017). Structural inheritance of the actin cytoskeletal organization determines the body axis in regenerating Hydra. *Cell reports*, *18*(6), 1410-1421.
(3) Bode, H. (2011). Axis formation in hydra. *Annual review of genetics*, *45*, 105-117.
(4) Braun, E., & Keren, K. (2018). Hydra Regeneration: Closing the Loop with Mechanical Processes in Morphogenesis. *BioEssays*, 1700204.