Week 05: Animal cell mechanics

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Introduction

Animal cells do not have a cell wall

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Large deformation and ability to change shape

- Adapt to the environment
- Migration
- Cell division
- Exo and endocytosis
- Multicellular organization and tissue formation

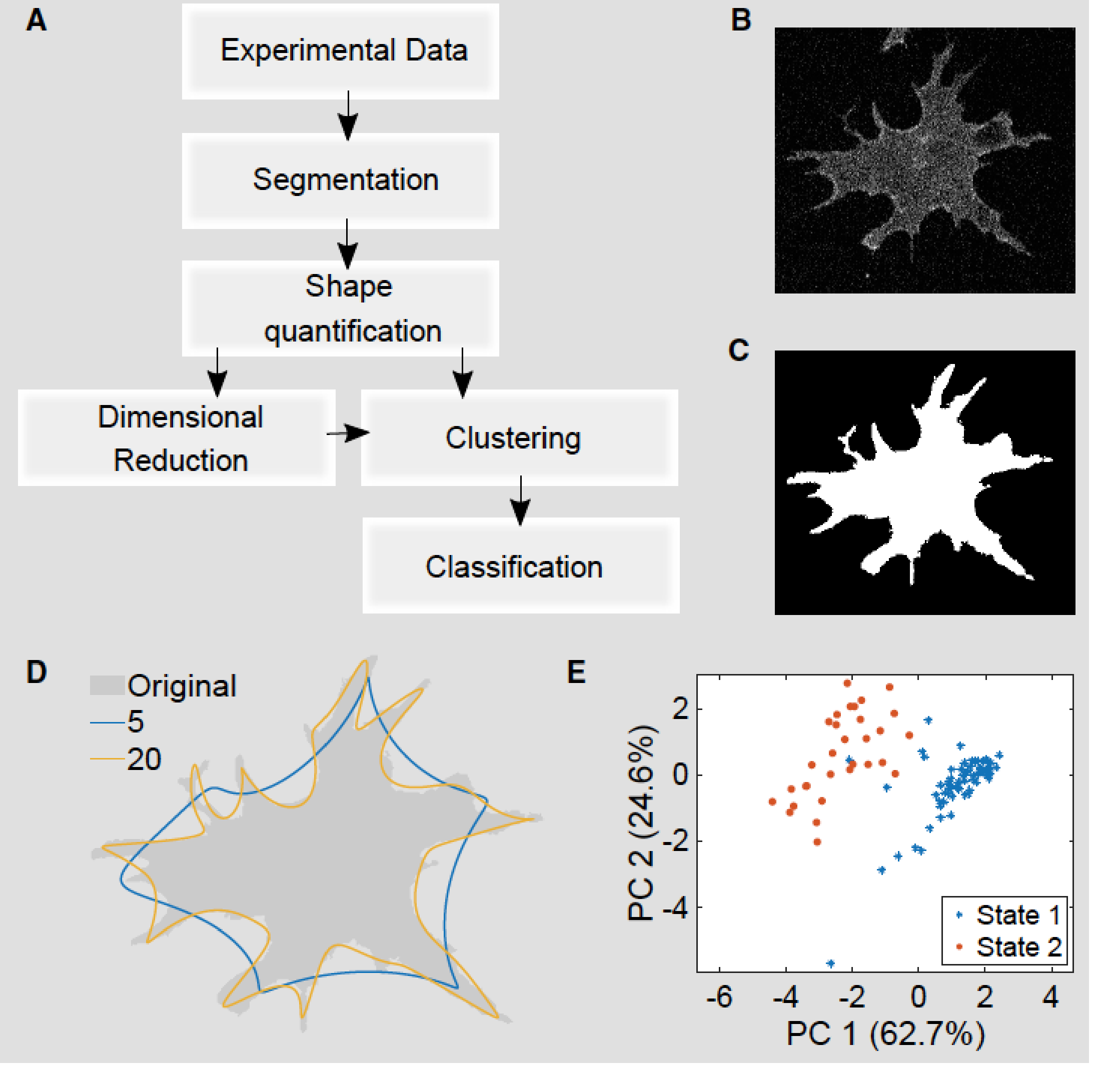
Force and structure

- Intracellular proteins

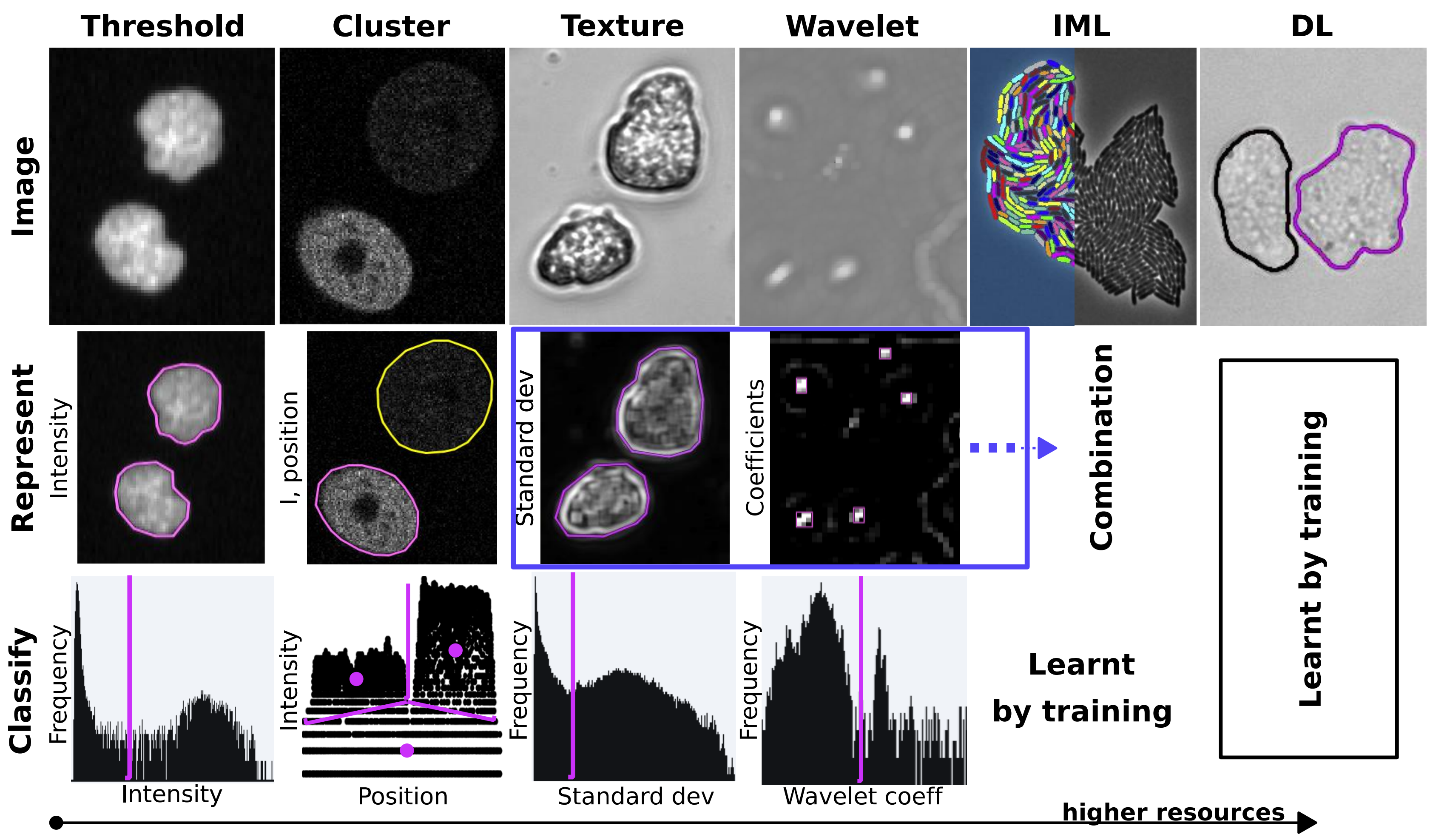
Cell morphometric analysis

- Image segmentation
  - Thresholding, active contour approach, classifier with annotations
- Surface reconstruction algorithms
- Quantification of cell shapes
  - Area, perimeter, circularity, volume, surface area
  - Landmark points: protrusions
  - Fourier descriptors (complex shapes)
  - Moment invariants: summing up pixels with weigh functions
- Dimensional reduction methods
  - Principal component analysis
  - Support vectors
  - Decision trees
- Deep learning methods

Cell morphometric analysis

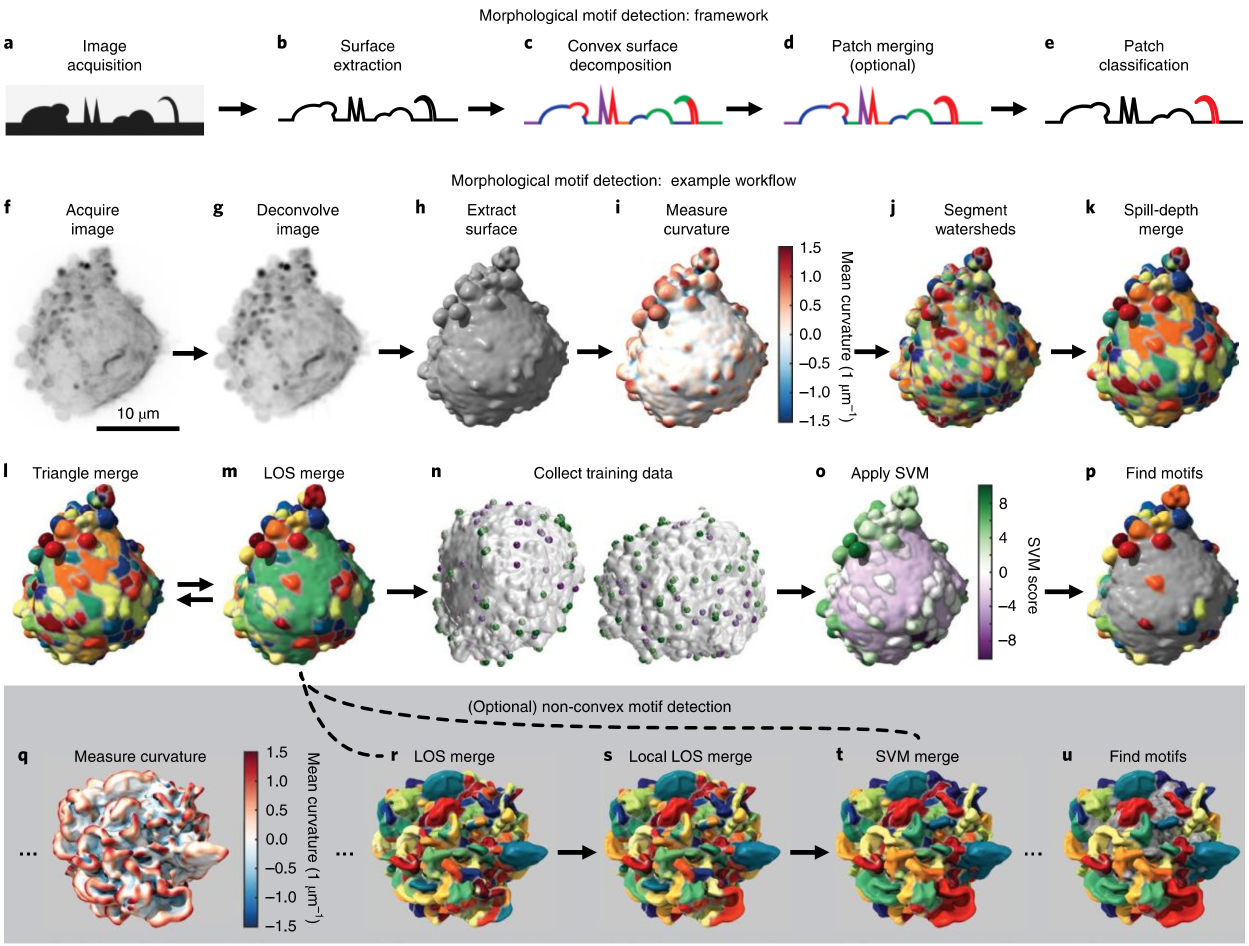


Cell morphometric analysis



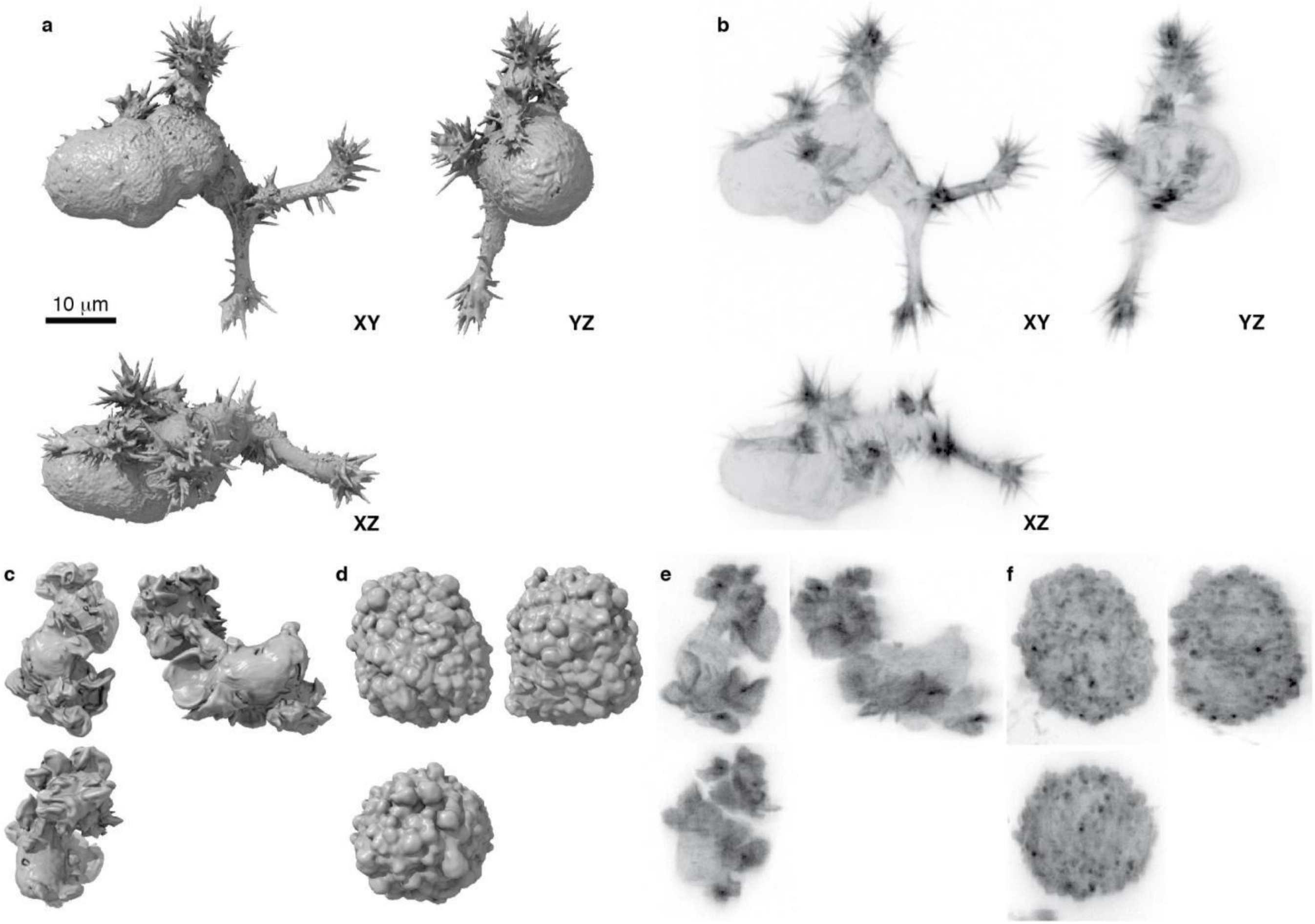
Automated detection of morphological motifs

- Computer graphics and machine learning pipeline



Automated detection of morphological motifs

- Laser scanning confocal and light sheet microscopy



Automated detection of morphological motifs

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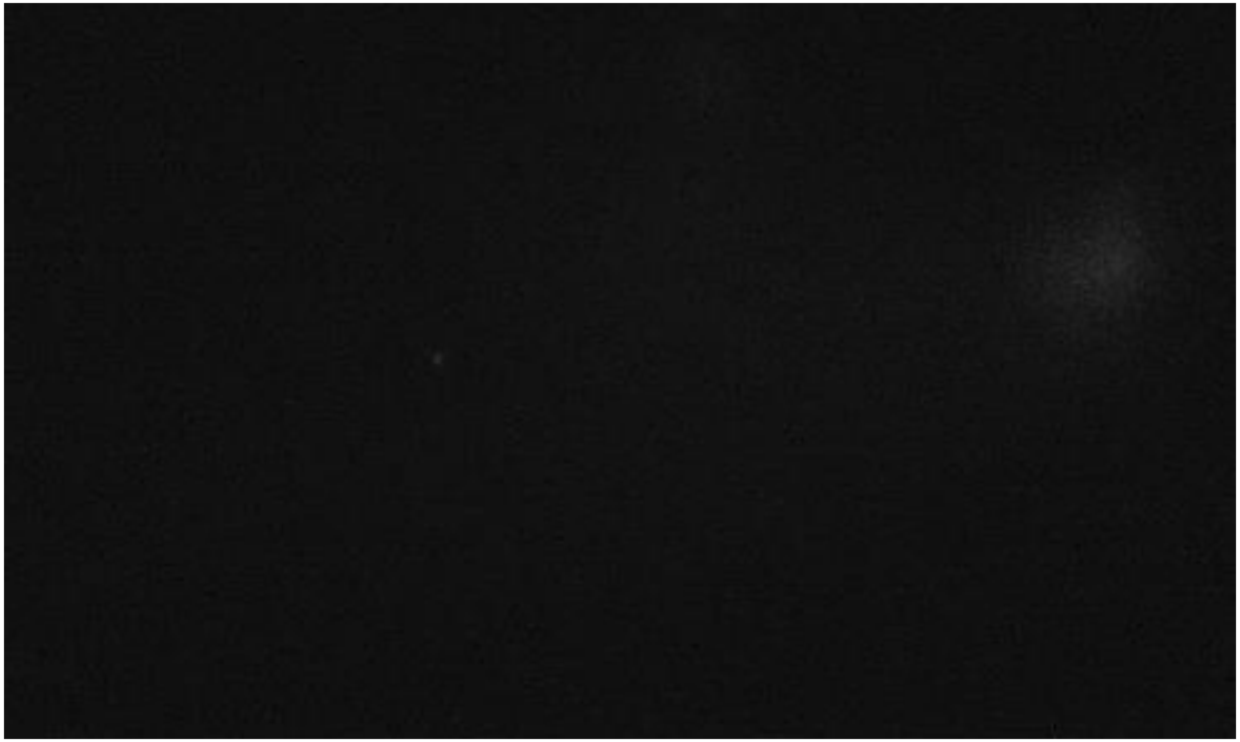
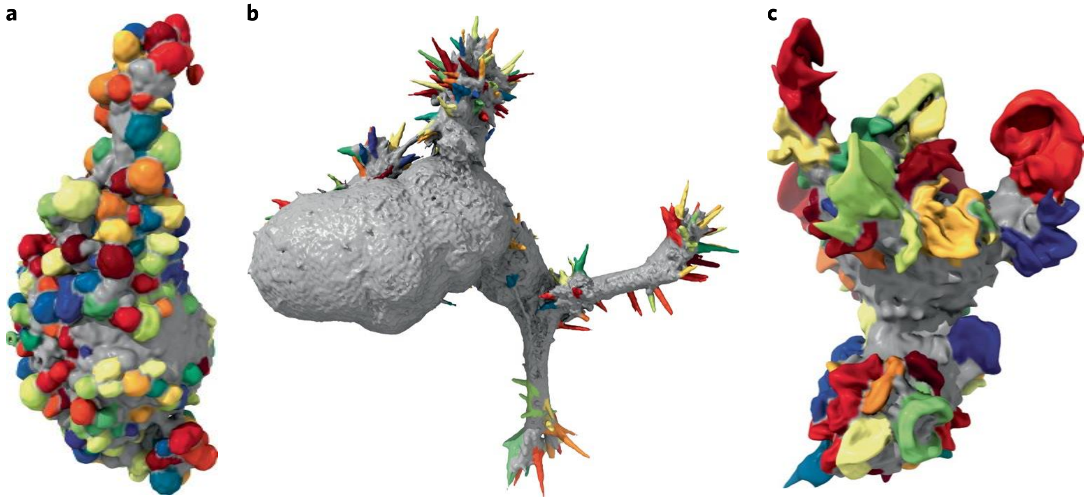


Automated detection of morphological motifs

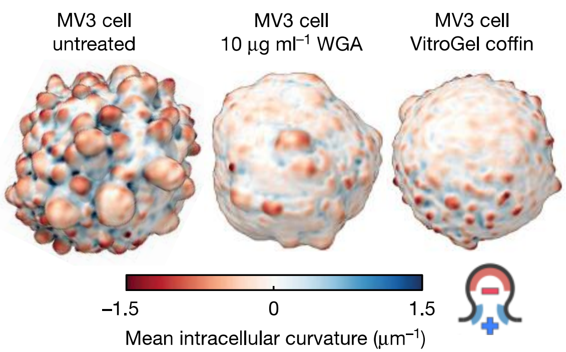
blebs filapodia lamellipodia

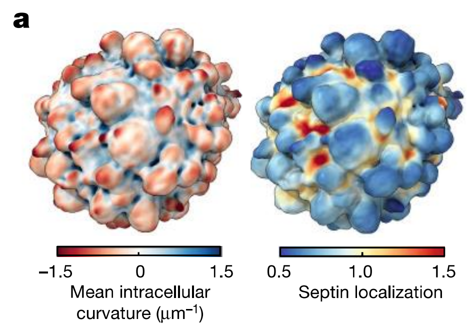


From morphology to signaling to therapy

Blebs promote cell survival by assembling oncogenic signalling hubs

Andrew D. Weems[1✉], Erik S. Welf[1,2], Meghan K. Driscoll[1,3], Felix Y. Zhou[1], Hanieh Mazloom-Farsibaf[1], Bo-Jui Chang[1], Vasanth S. Murali[1], Gabriel M. Gihana[1], Byron G. Weiss[1], Joseph Chi[1], Divya Rajendran[1], Kevin M. Dean[1], Reto Fiolka[1] & Gaudenz Danuser[1✉]





- Most human cells require anchorage for survival. Cell–substrate adhesion activates diverse signalling pathways, without which cells undergo anoikis—a form of programmed cell death. Acquisition of anoikis resistance is a pivotal step in cancer disease progression, as metastasizing cells often lose firm attachment to surrounding tissue. In these poorly attached states, cells adopt rounded morphologies and form small hemispherical plasma membrane protrusions called blebs. Blebbing triggers the formation of plasma membrane-proximal signalling hubs that confer anoikis resistance. Inhibition of blebs or septins has little effect on the survival of well-adhered cells, but in detached cells it causes NRAS mislocalization, reduced MAPK and PI3K activity, and ultimately, death.

Cell Theory

- First formulated in mid-nineteenth century
  - All living organisms are made up of cells
  - The cell is the basic structural and organizational unit
  - Cells arise from pre-existing cells

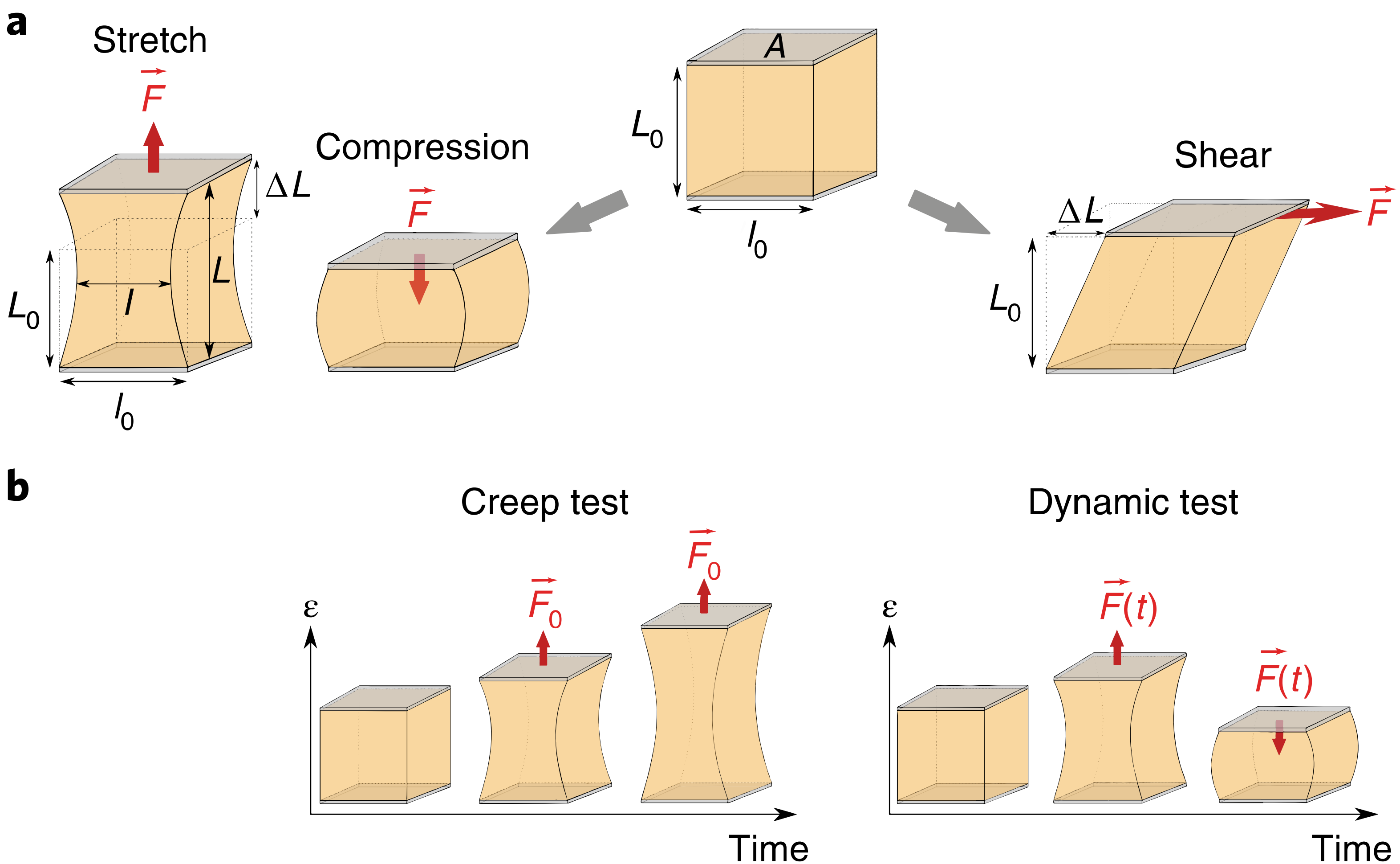
- Invention of microscope (Hooke, van Leuwenhoek)
- Schwann and Schleiden

- Modern interpretation
  - Energy flow occurs within cells
  - Heredity information (DNA) is passed on from cell to cell
  - All cells have the same chemical composition

Material structure vs functional responses under load

- Materials can be categorized by how they deform (or change shape) in response to mechanical loading, typically in a stress-strain test.
- Mechanical stress is defined as the force per unit area, with units of pascals (newtons per square meter) and can be in shear or normal.
- Strain is a normalized measure of deformation.
- Constitutive equations describe the relationship between stress and strain for a given material.
- Biological tissues and ECMs can exhibit a combination of nonlinear elasticity, viscoelasticity, poroelasticity and plasticity. Materials that are both viscoelastic and plastic are considered to be viscoplastic.

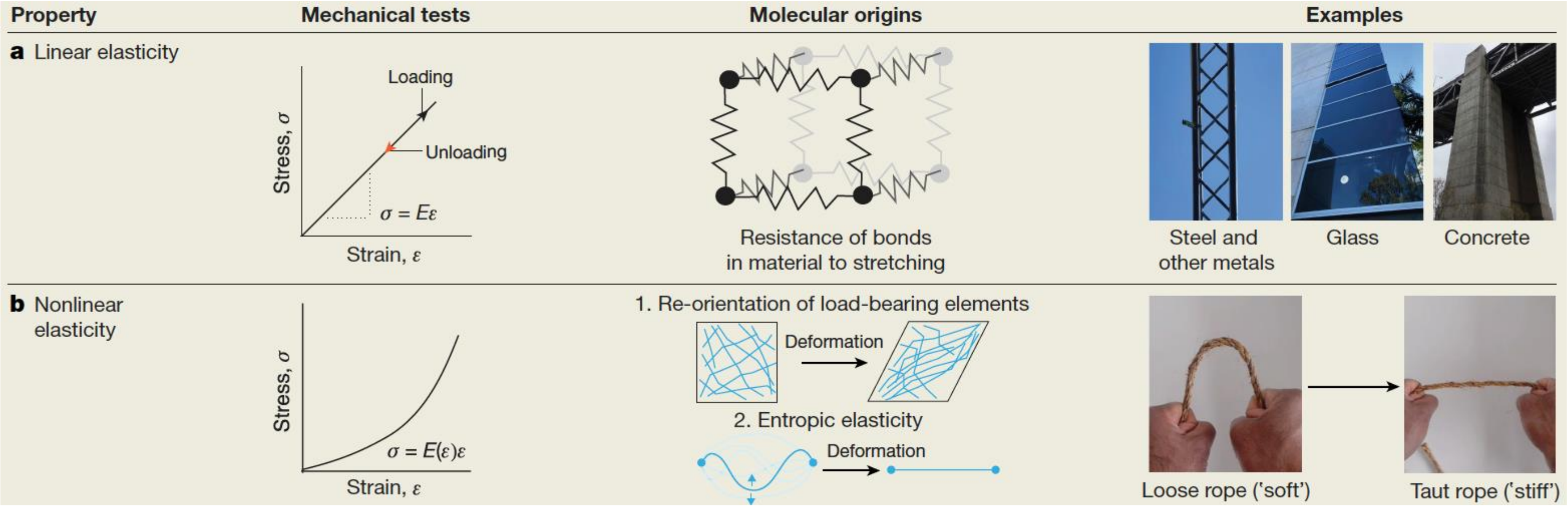
Rheological tests



Mechanics of living matter

- Tissues appear to be macroscopically solid
- Behave very differently compared to perfectly elastic or Hookean solids when put under pressure or stretched

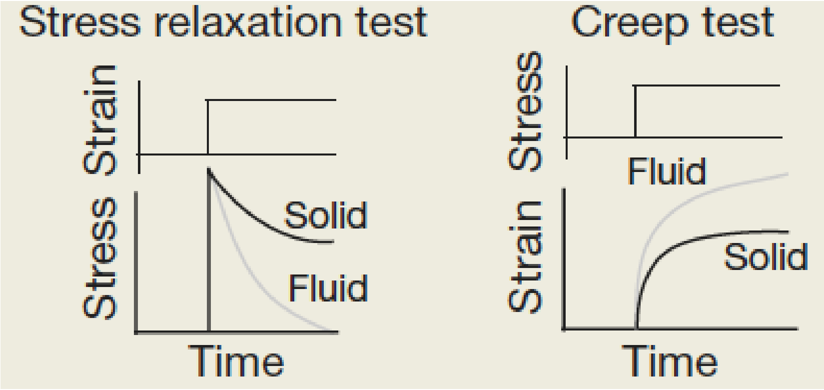
- Time-dependent and strain-dependent mechanical responses



Viscoelasticity

- In response to a mechanical perturbation, viscoelastic materials exhibit an instantaneous elastic response, which is characteristic of purely elastic solids, followed by a time-dependent mechanical response and **energy dissipation or loss**, both of which characteristic are of viscous liquids.

- Viscoelastic materials will '**creep**', or deform in a time-dependent manner, in response to the application of an external step stress or load, and undergo '**stress relaxation**', or reduce stress levels in a time-dependent manner, in response to a step deformation.



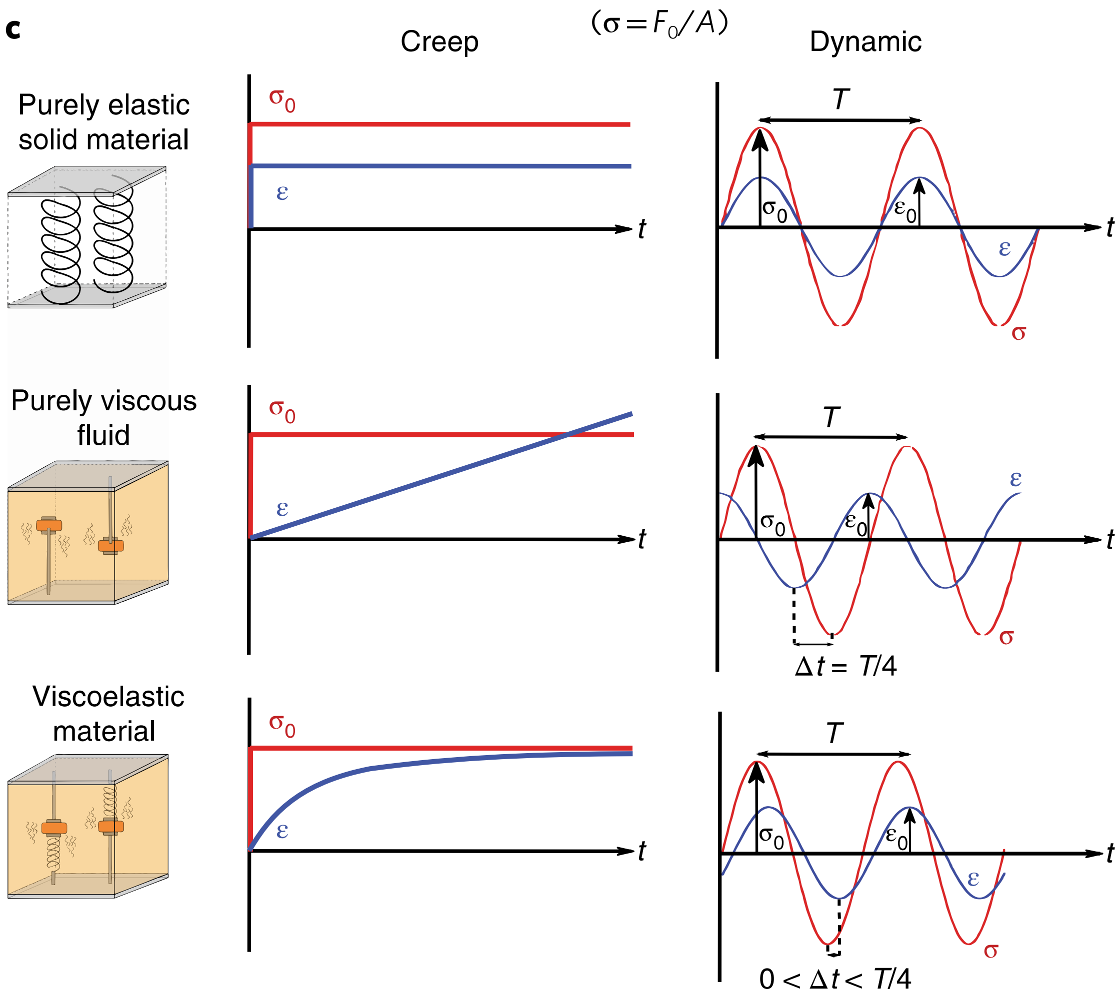
Viscoelasticity

- Under an imposed sinusoidal deformation, stress and strain are completely in-phase for a purely elastic material, because all of the input deformation energy can be ‘stored’ and ‘recovered’ during each cycle without any loss.

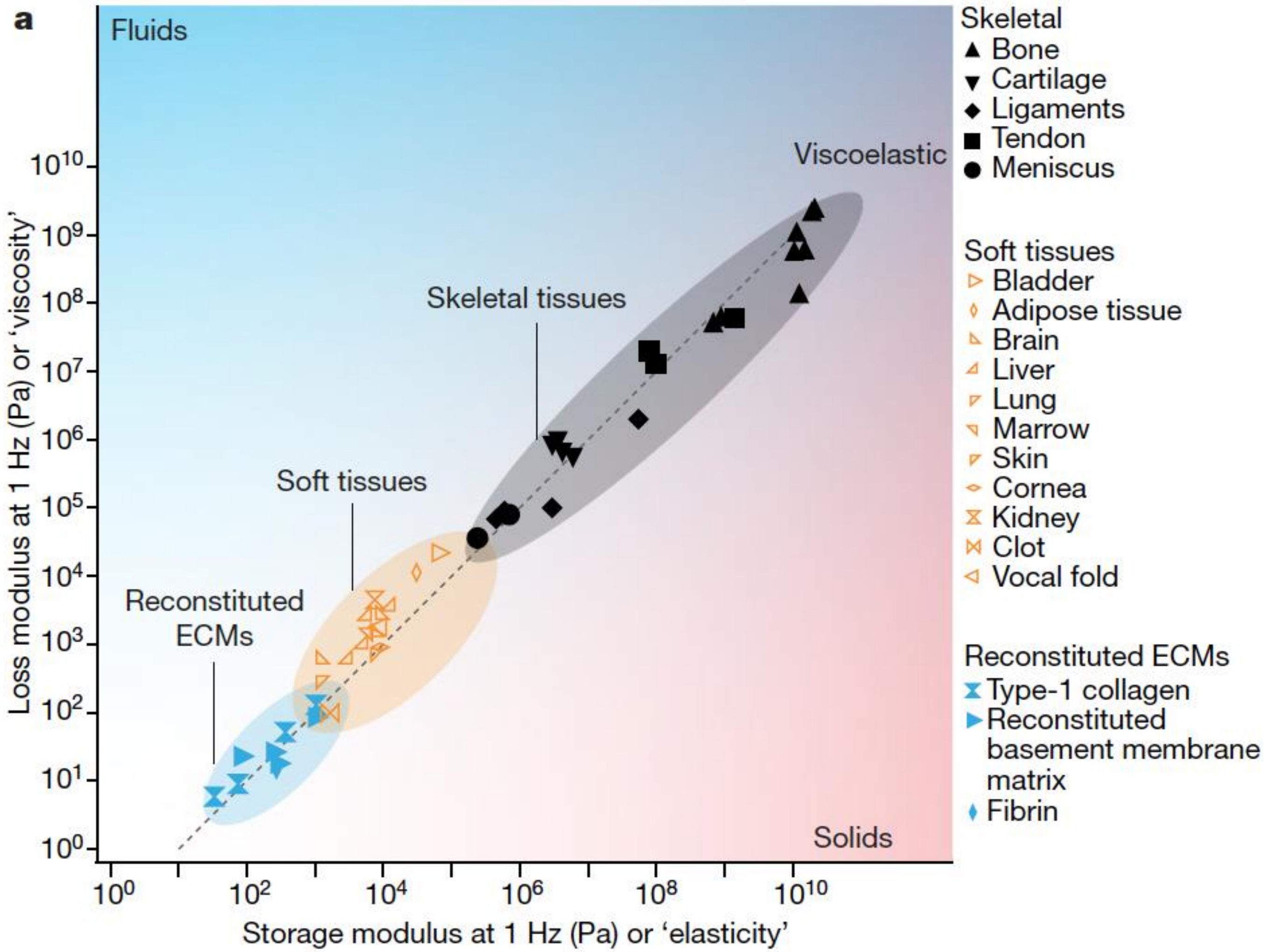
- Whereas for a purely viscous fluid they are completely out-of-phase, a result of all of the input deformation energy being dissipated or ‘lost’ by internal friction in the system as it flows.

- Viscoelastic materials exhibit a response between these two extremes, with the in-phase component of the response described as the **storage (or elastic) modulus** and the out-of-phase response described as the **loss (or viscous) modulus**.

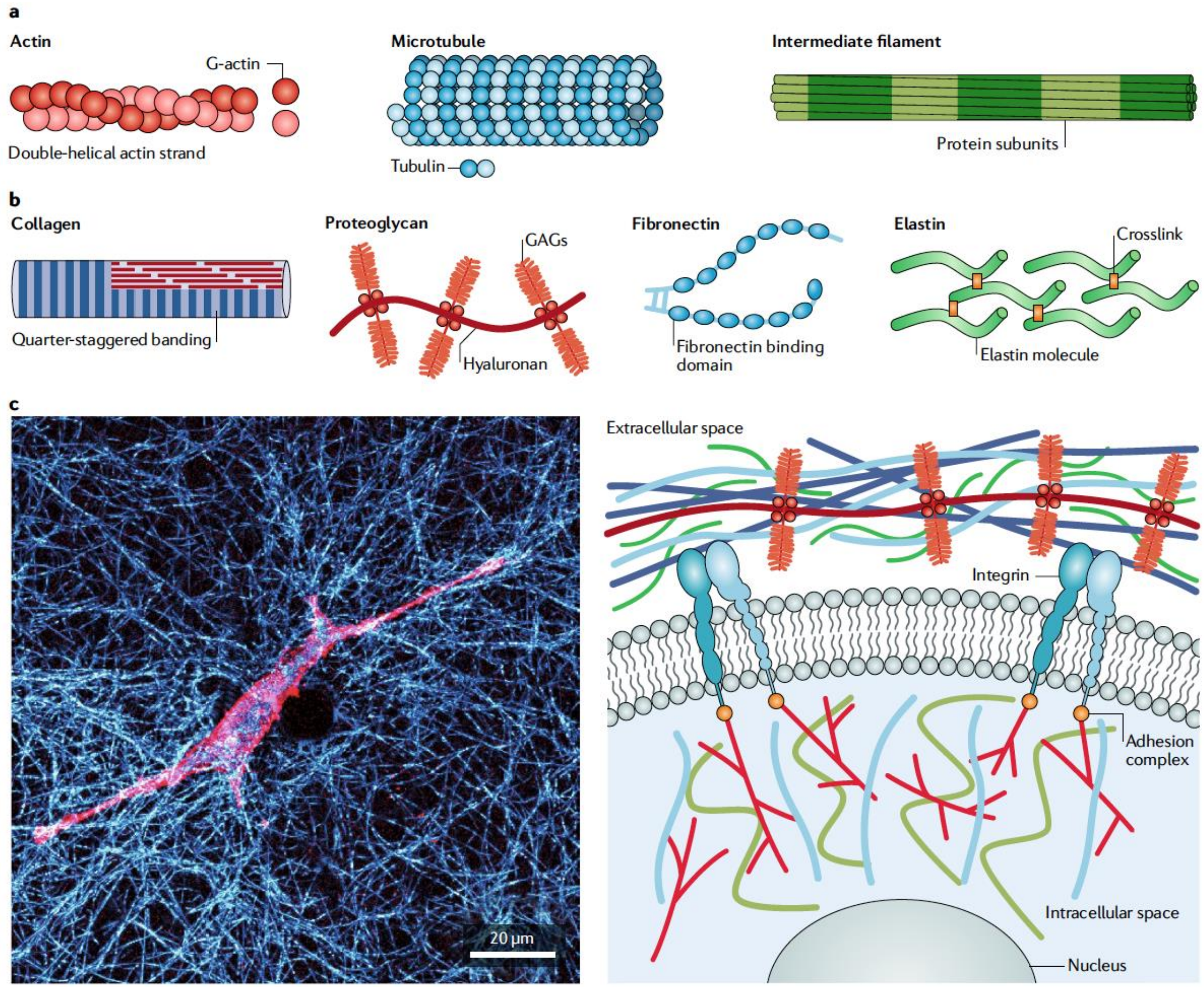
Viscoelasticity



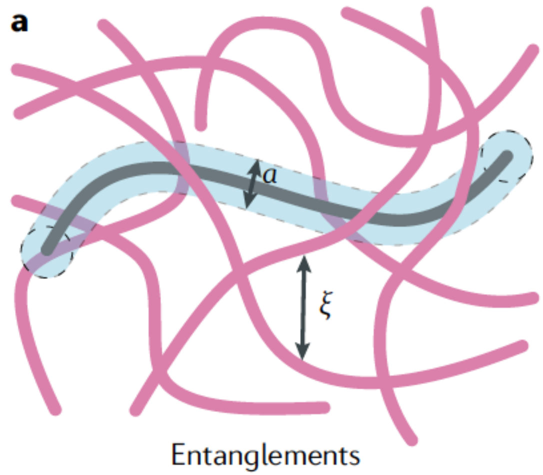
Viscoelasticity



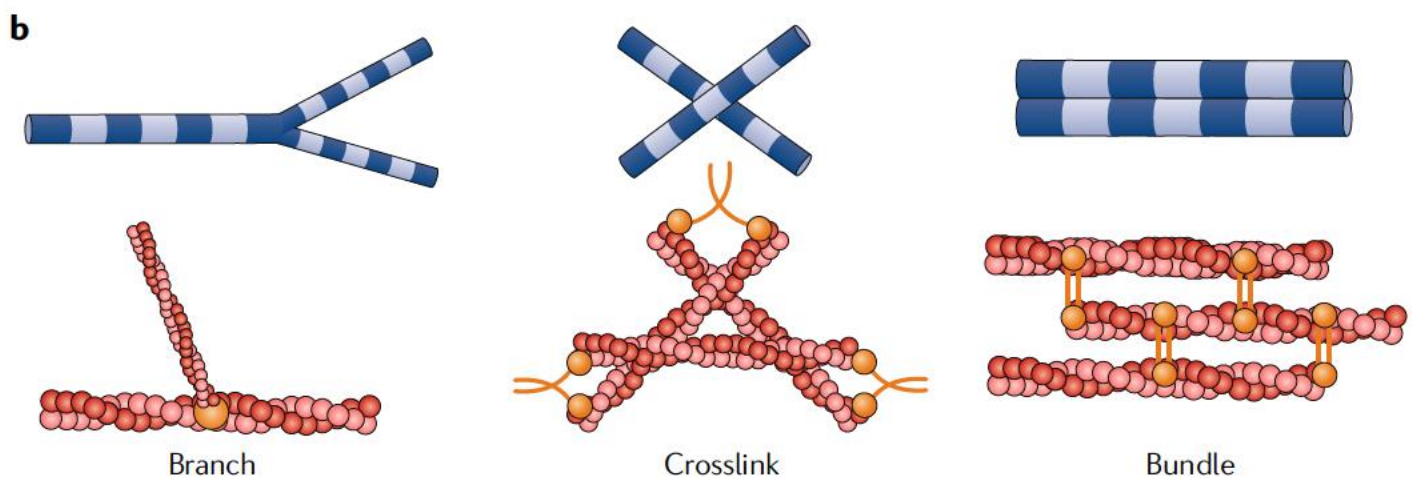
Biopolymer networks



Mechanisms of forming load-bearing networks



High density + steric interactions
Storing elastic energy on short timescales

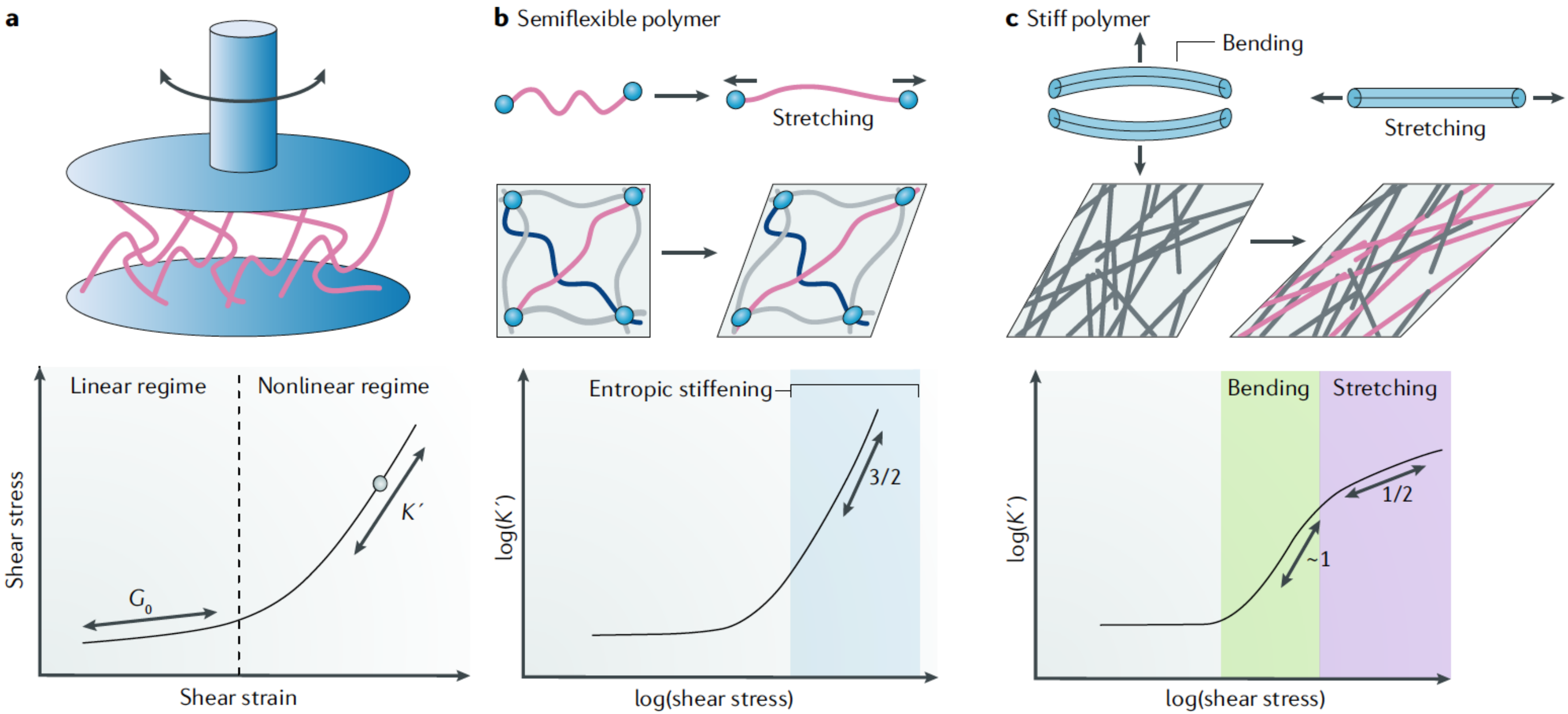


Collagen

Actin

Nonlinear elasticity in biopolymer networks

- Stress-stiffening in response to shear or uniaxial tensile loads
- Stress-softening under compressive loading (buckling)
- Derivative of the stress–strain curve gives the differential modulus (K')



Nonlinear elasticity in biopolymer networks

- Networks of actin and intermediate filaments are **highly sensitive to strain**
  - A few percent strain leads to an increase of stiffness by a factor of 10-100
- Conformational entropy decreases as they are pulled along the principal strain

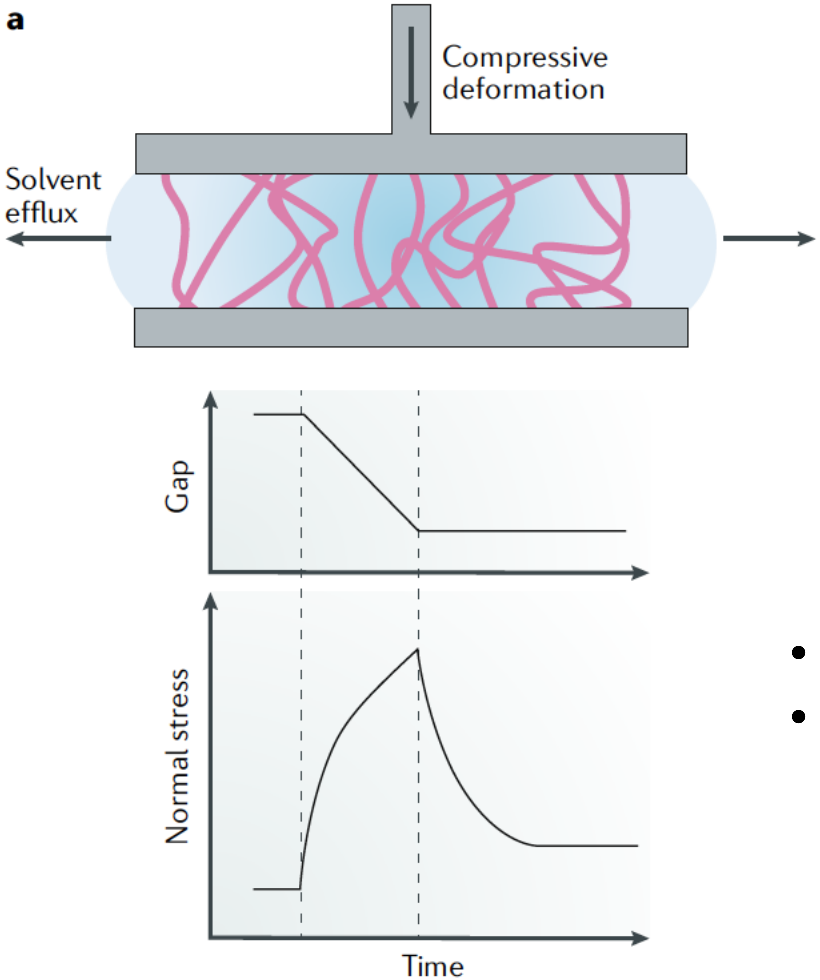
- Why?
  - Mechanically protects cells by preventing large deformations
  - Enables cells to tune their stiffness by molecular motor activity

Nonlinear elasticity in biopolymer networks

- Networks of stiff filaments such as collagen strain-stiffen due to network connectivity
  - Average coordination number is 3 to 4
  - Below Maxwell criterion (6) required for the stability of networks of springs
  - Stiffening starts at 10% strain and the stiffness can increase up to 100-fold

- Why?
  - Prevents tissue rupture
  - Promotes long range mechanical communication between cells

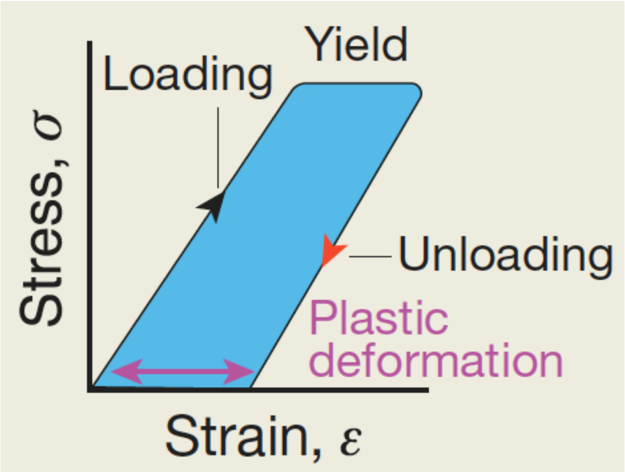
Poroelasticity of biopolymer networks

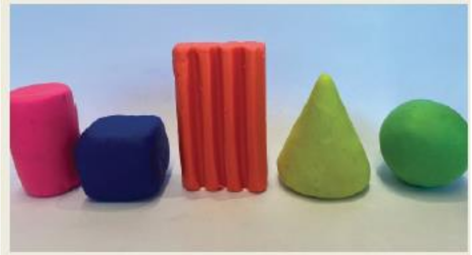


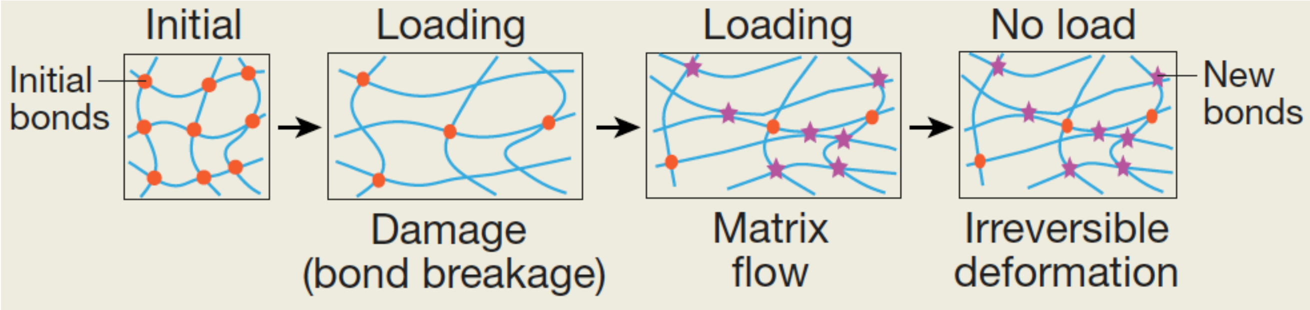
- The flow of water within ECM
- Local contraction of the cytoskeleton can lead to intracellular poroelastic effects and transient pressure gradients

Plasticity of biopolymer networks and tissues

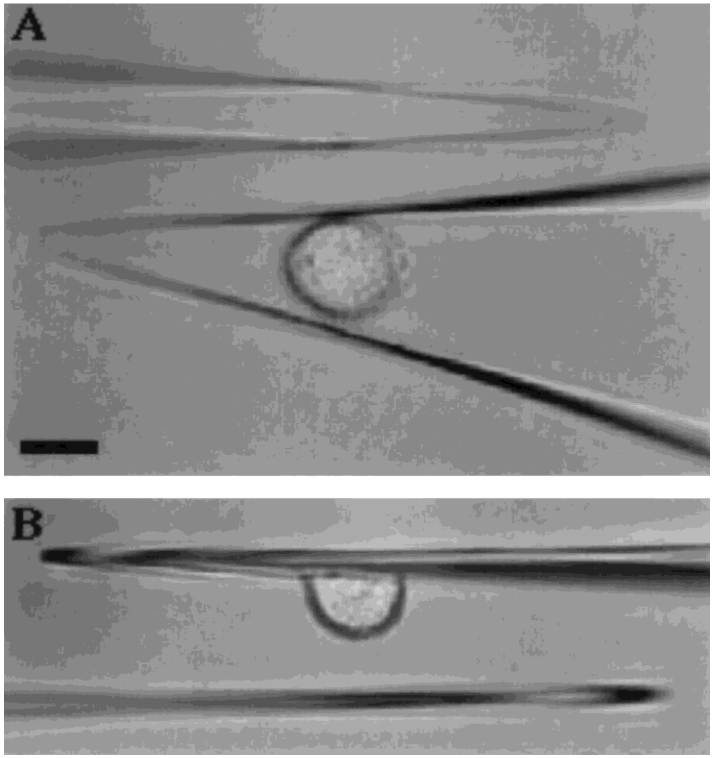
- Weak crosslinks and load-dependent dynamics
- Entanglement

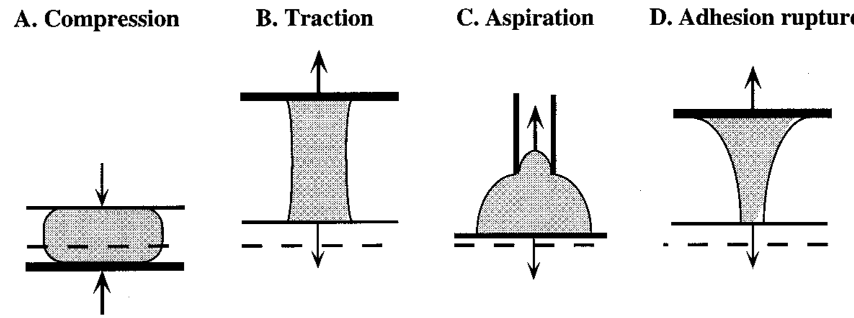


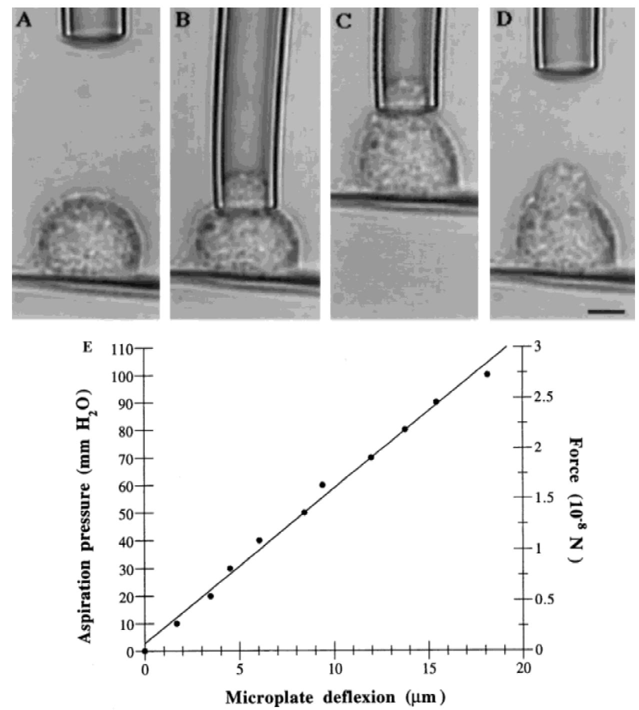




Glass microplates and micropipettes

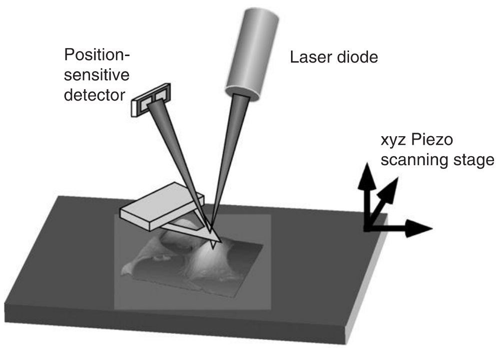


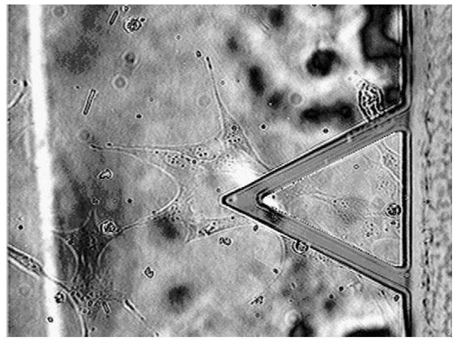


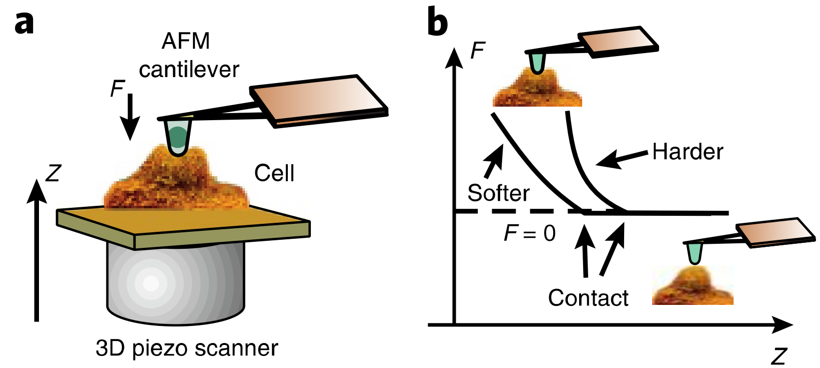


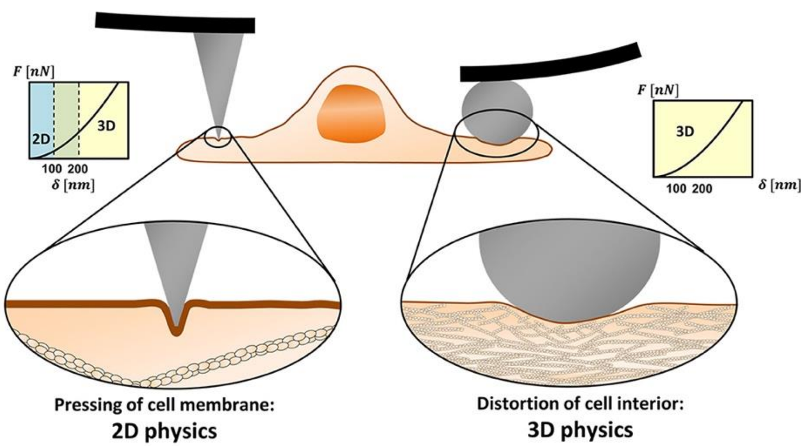
- Forces in the range of 1 nN to 1 µN

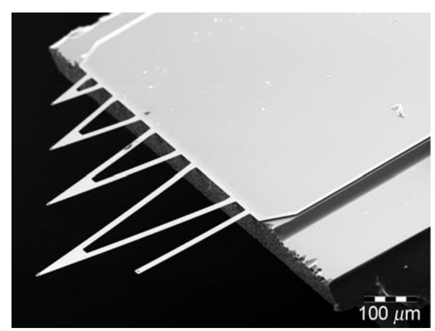
Atomic Force Microscope (AFM)

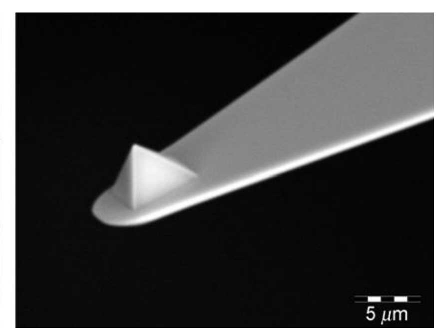












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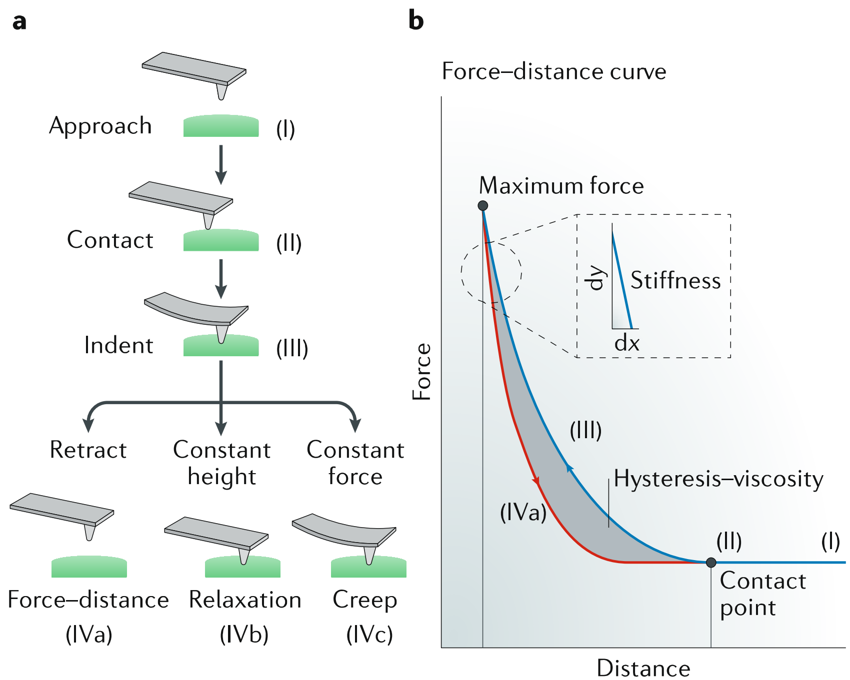
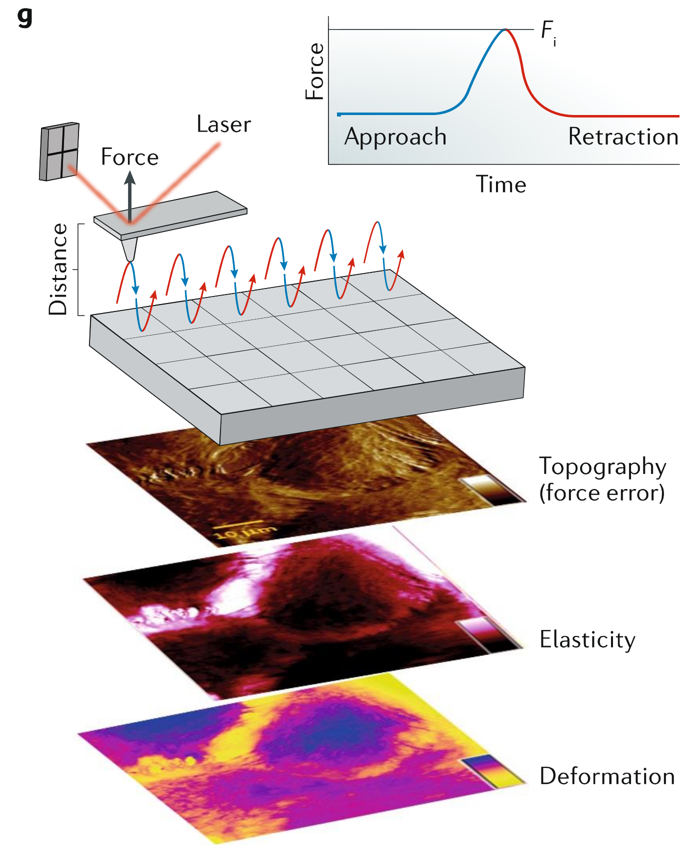
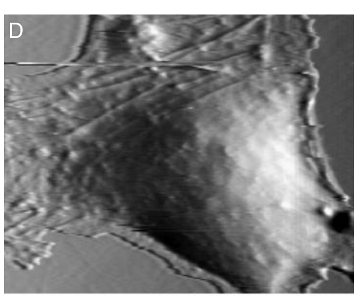
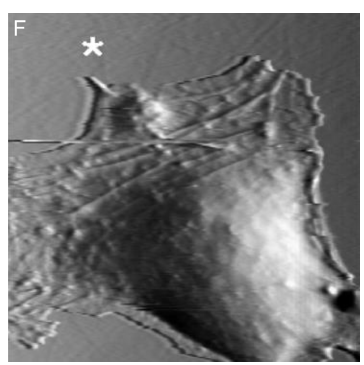


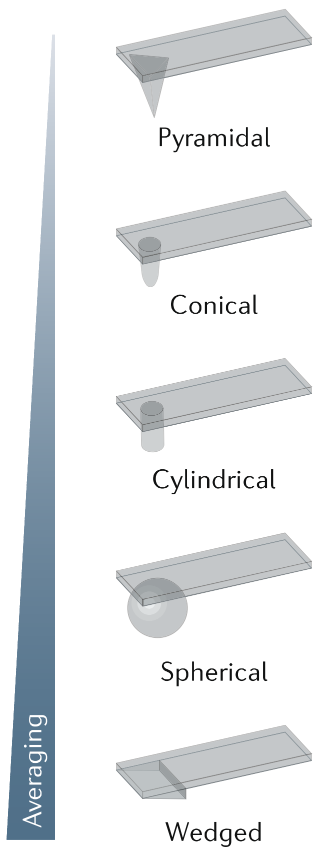


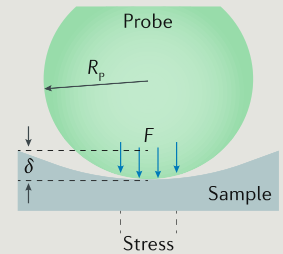




Basic contact models to calculate modulus

e



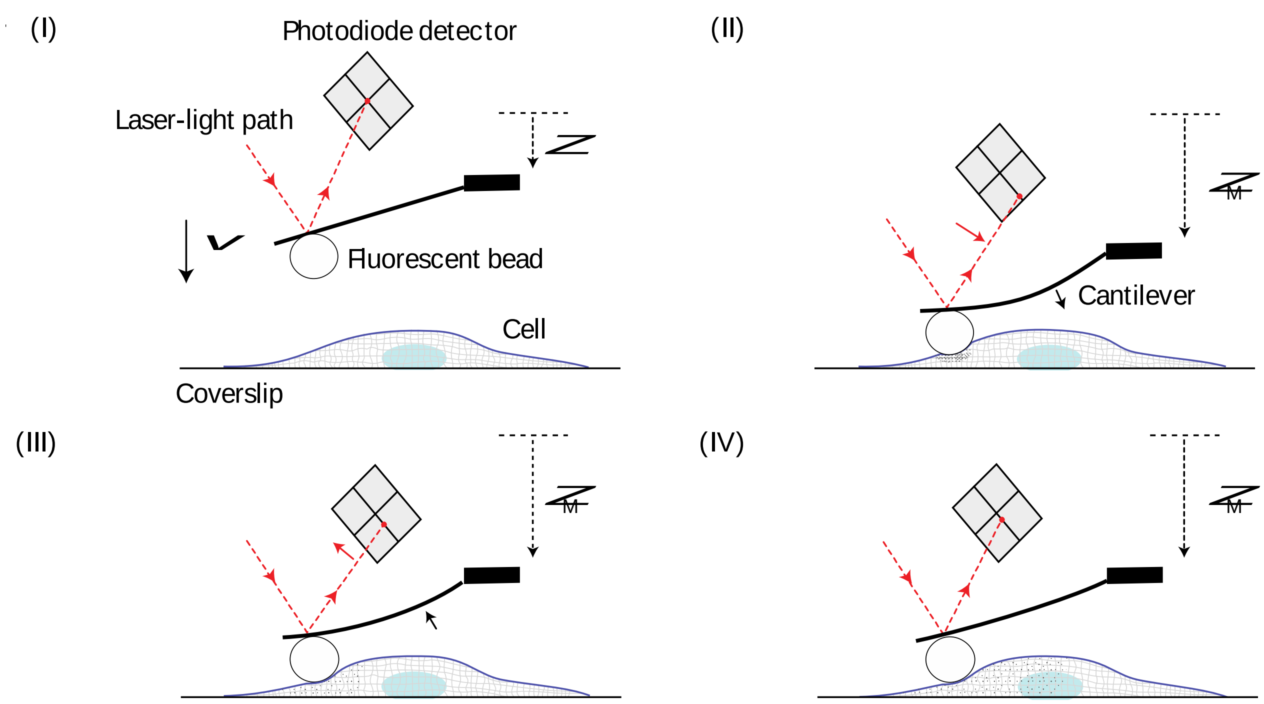
Model	Probe geometry	Force	Additional assumptions	Schematic representation
Hertz	Spherical	$F = E_{\mathrm{eff}} \cdot \left[\left(a^2 + R_P^2\right) \cdot \ln\left(\frac{R_P + a}{R_P - a}\right) - 2aR_P\right]$ $\delta = \frac{a}{2} \ln \frac{R_P + a}{R_P - a}$	No surface forces	
	Cylindrical	$F(\delta) = 2E_{\mathrm{eff}} \cdot R_Z \delta$	Smooth punch profile (no edges)	–
	Conical (Sneddon model)	$F(\delta) = E_{\mathrm{eff}} \cdot 2\tan(\theta)/\pi \cdot \delta^2$	Infinitely sharp probe	–
	Parabolic	$F(\delta) = E_{\mathrm{eff}} \cdot \frac{4\sqrt{R_P}}{3} \delta^{\frac{3}{2}}$	$R_C > \delta$	–
	Blunted pyramidal	$F(\delta) = 2E_{\mathrm{eff}} \cdot \left[\delta a - \frac{\sqrt{2}}{\pi} \frac{a^2}{\tan\theta}\left(\frac{\pi}{2} - \arcsin\frac{b}{a}\right) - \frac{a^3}{3R_P} + \sqrt{(a^2 - b^2)} \cdot \left(\frac{\sqrt{2}}{\pi} \frac{b}{\tan\theta} + \frac{a^2 - b^2}{3R_P}\right)\right]$	Cross section of pyramid modelled as a circle	–

δ, indentation; η, cytosolic viscosity; v, Poisson ratio; ξ, pore size; a, contact radius; b, transition radius[178] of a blunt probe

E, Young's modulus; $E_{\mathrm{eff}} = E/(1 - v^2)$, effective Young's modulus; F, indenting force

Cytoplasm behaves as a poroelastic material

- Sudden increase in the local stress and pressure inside the cell (II)
- Over time, the cytosol in the compressed area redistributes (III and IV)
- At equilibrium, the applied force is balanced by cellular elasticity
- Indentation → elastic properties, Relaxation → time-dependent properties



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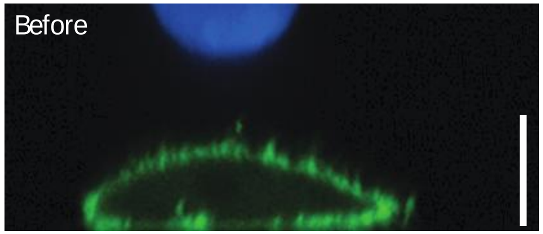
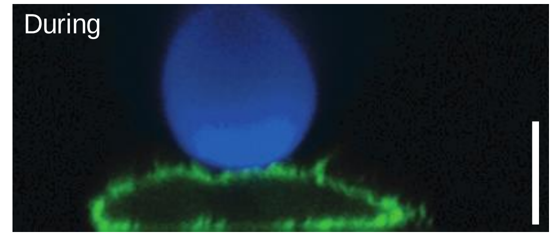
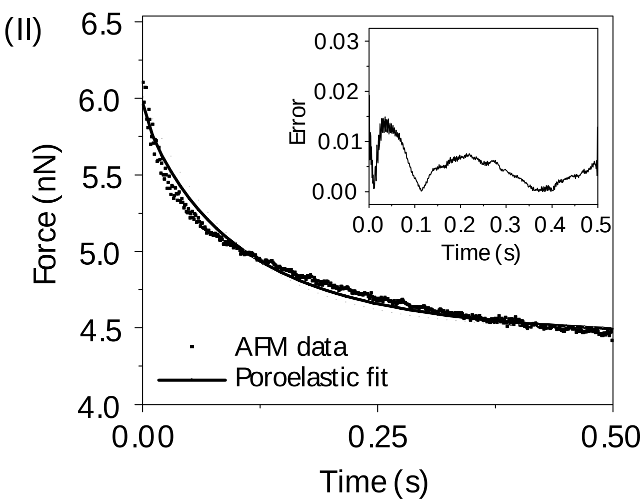
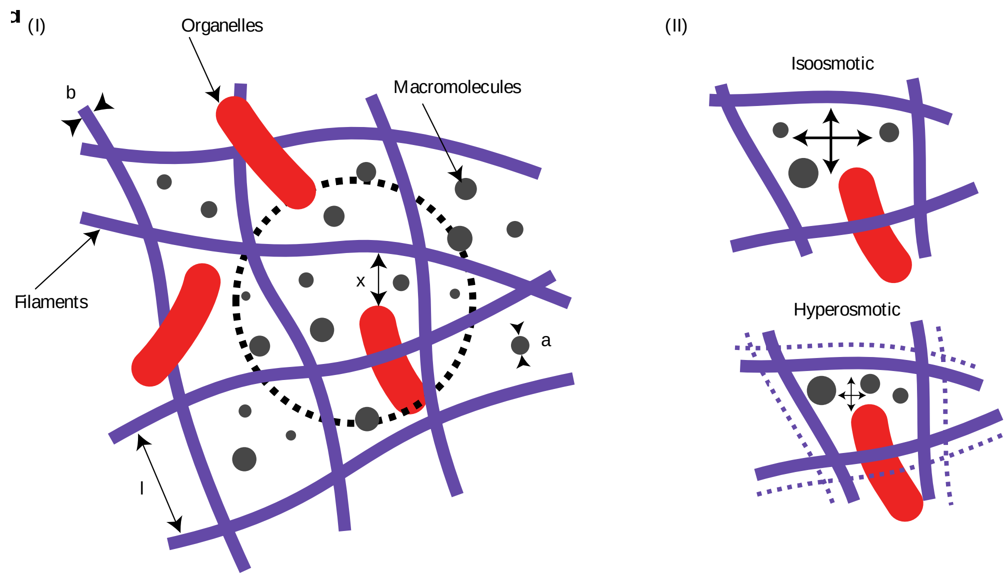


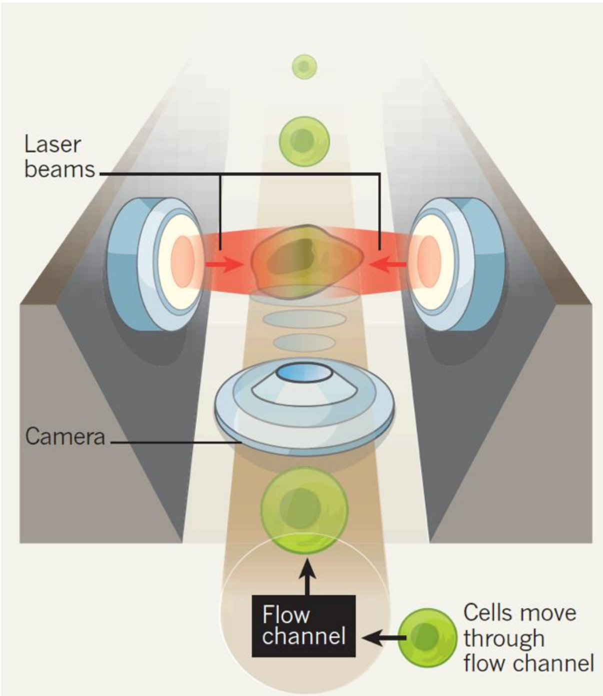


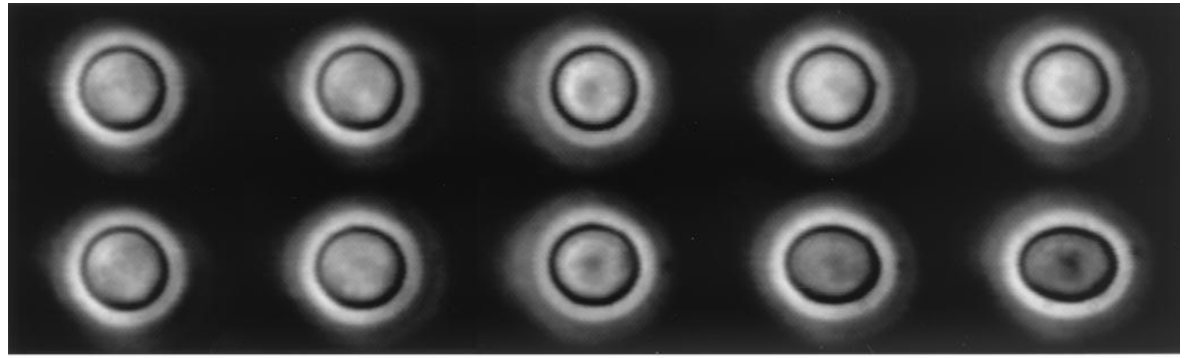


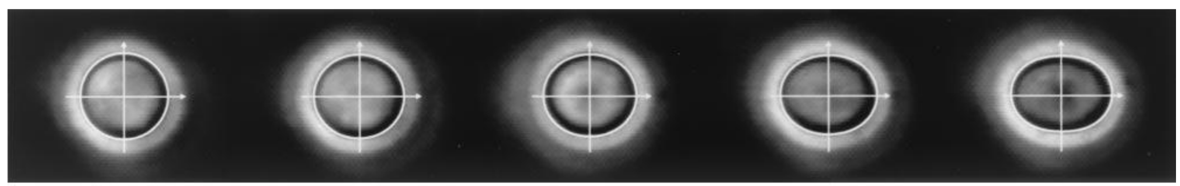


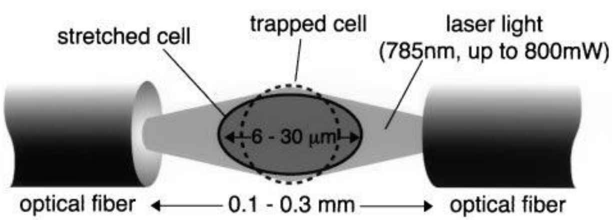
- Cytoskeleton and macromolecular crowding sets a hydraulic pore size
- Reduction in cell volume decreases entanglement length and increases crowding, which together lead to a decrease in hydraulic pore size

Optical stretching



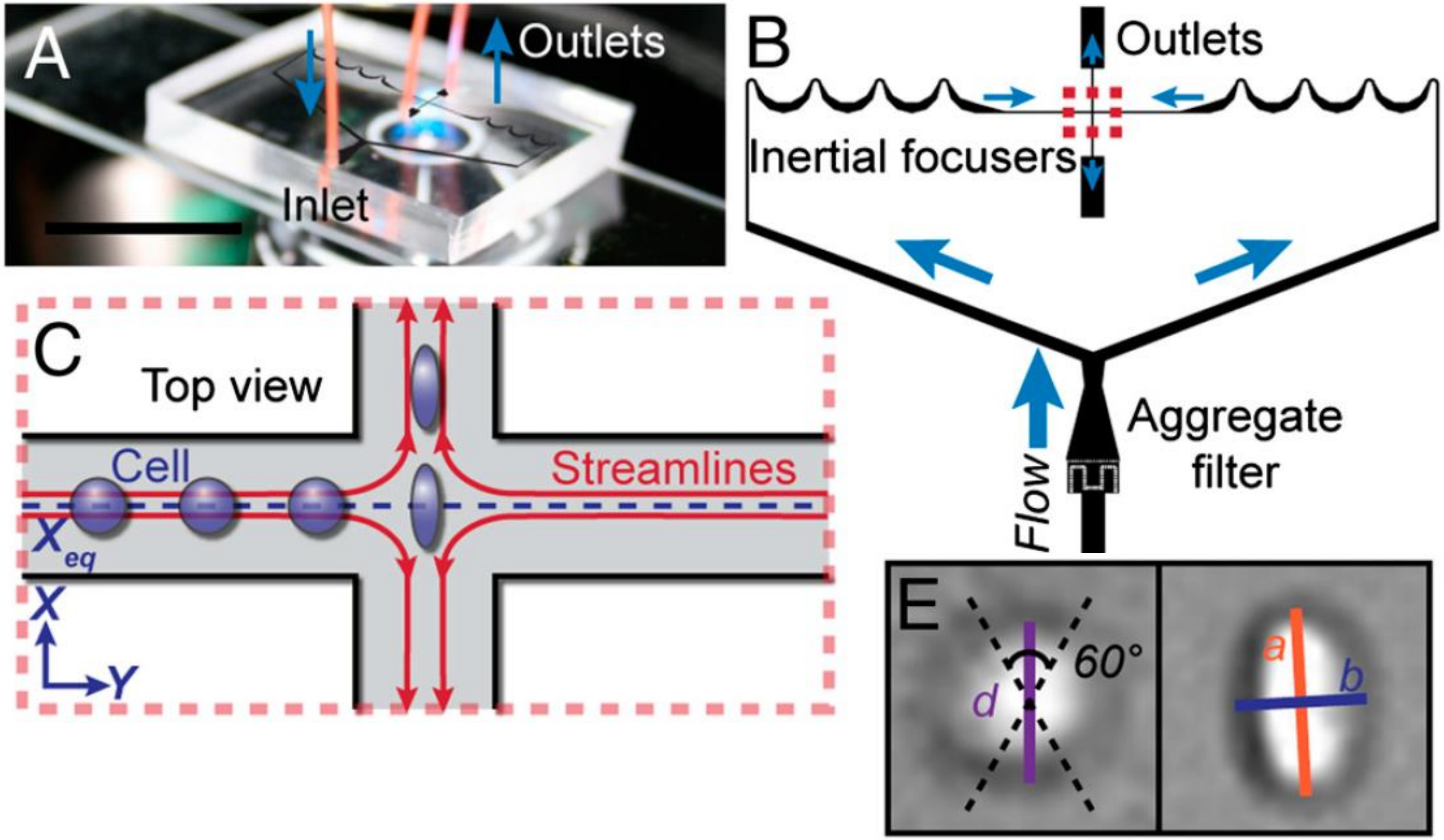






Hydrodynamic stretching

- Probing single cell deformability at 2,000 cells/s
- Inertial focusing and extensional flow



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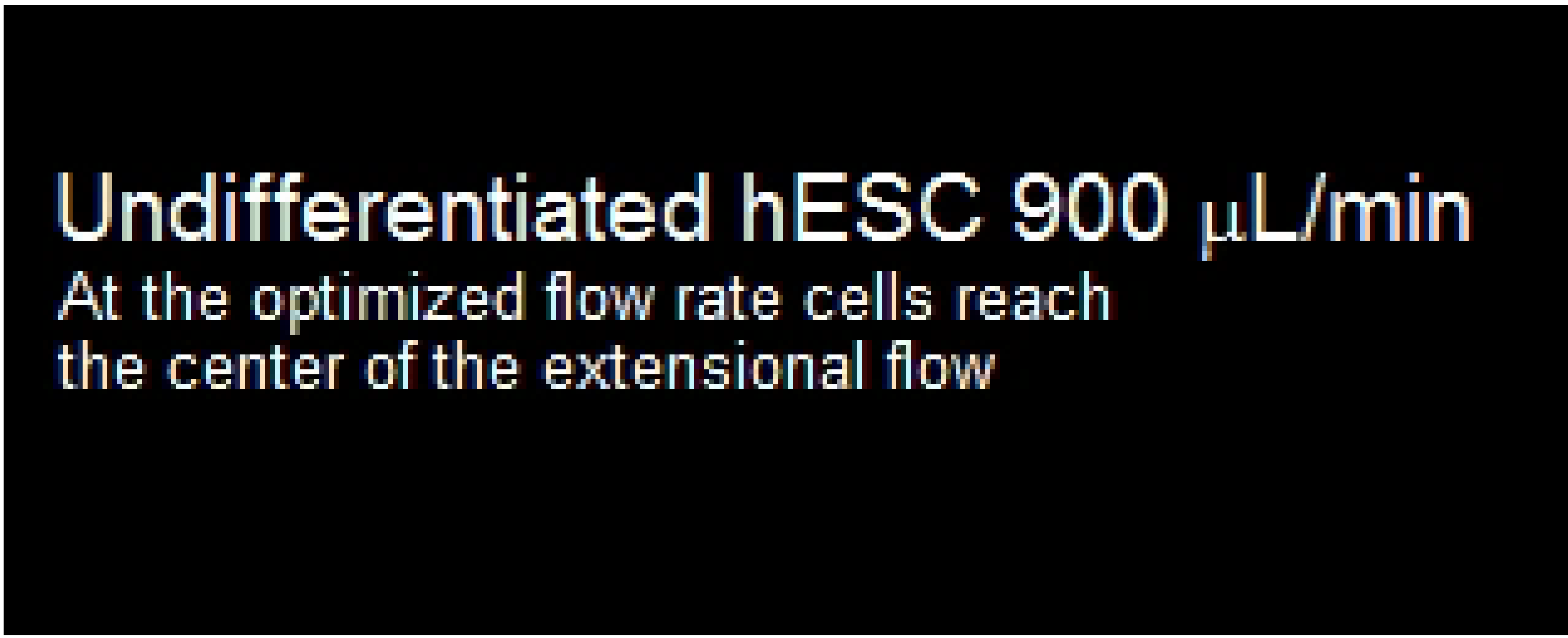
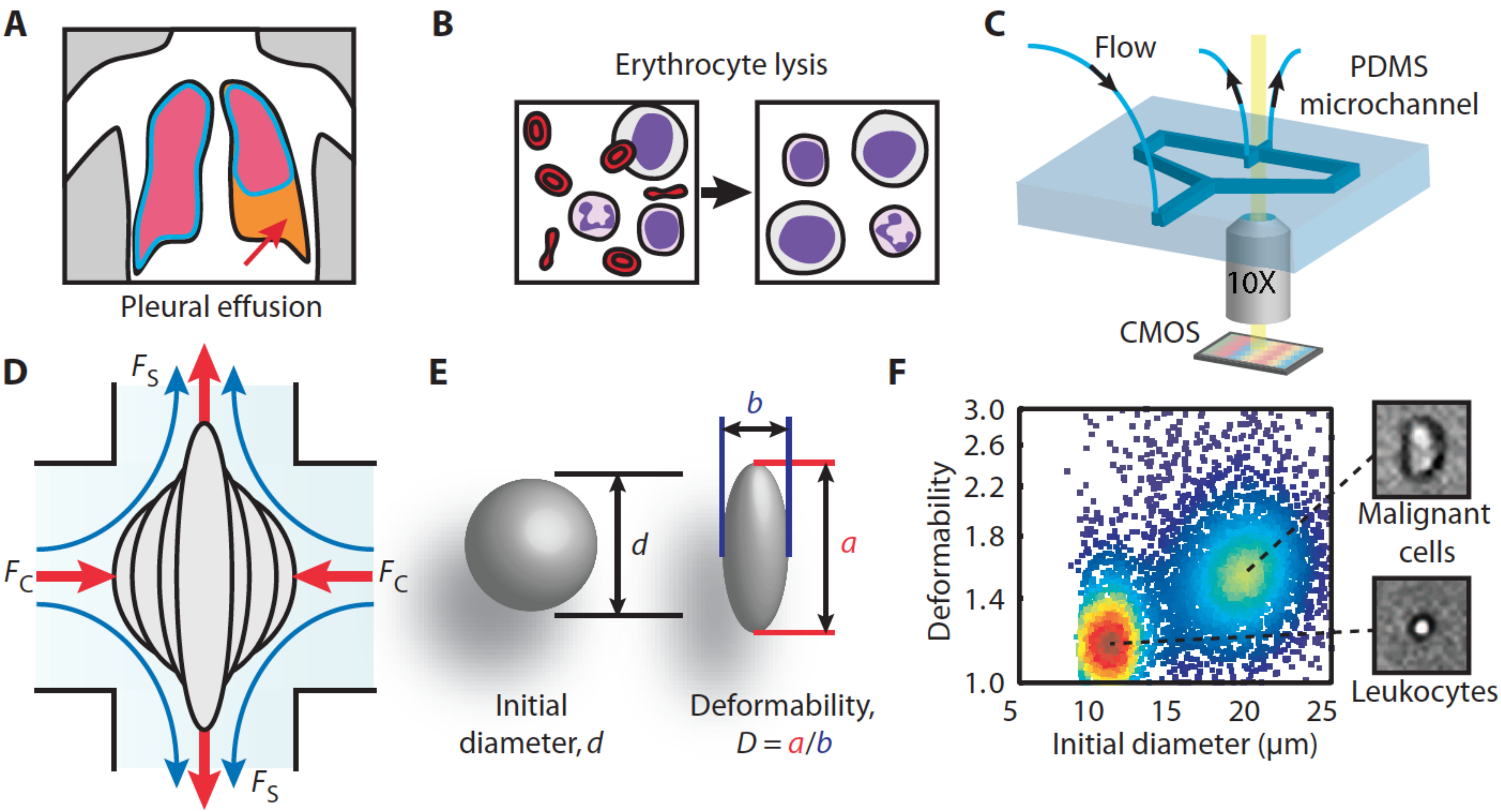
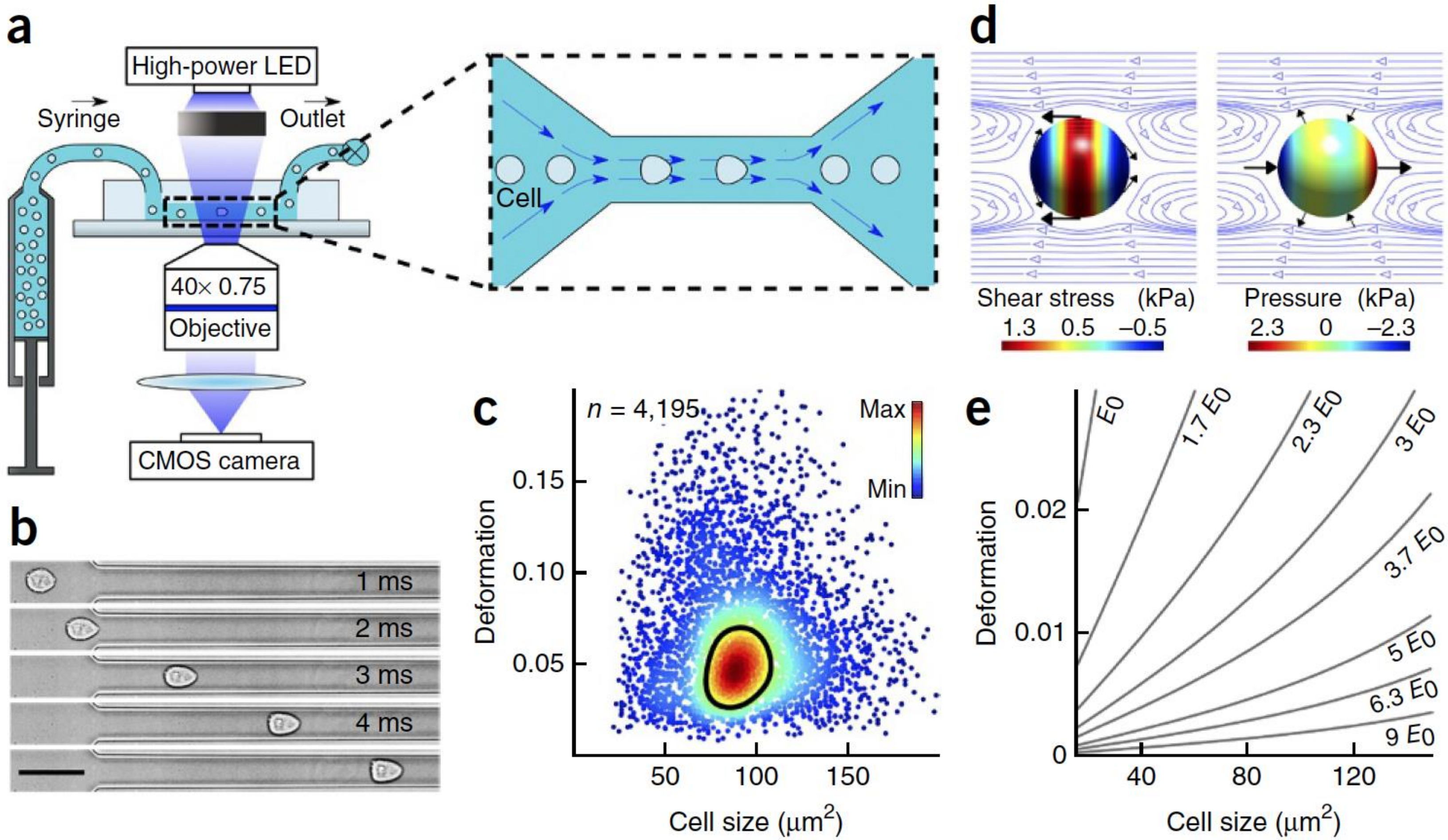


Mechanical profiling of pleural effusions for cancer diagnosis



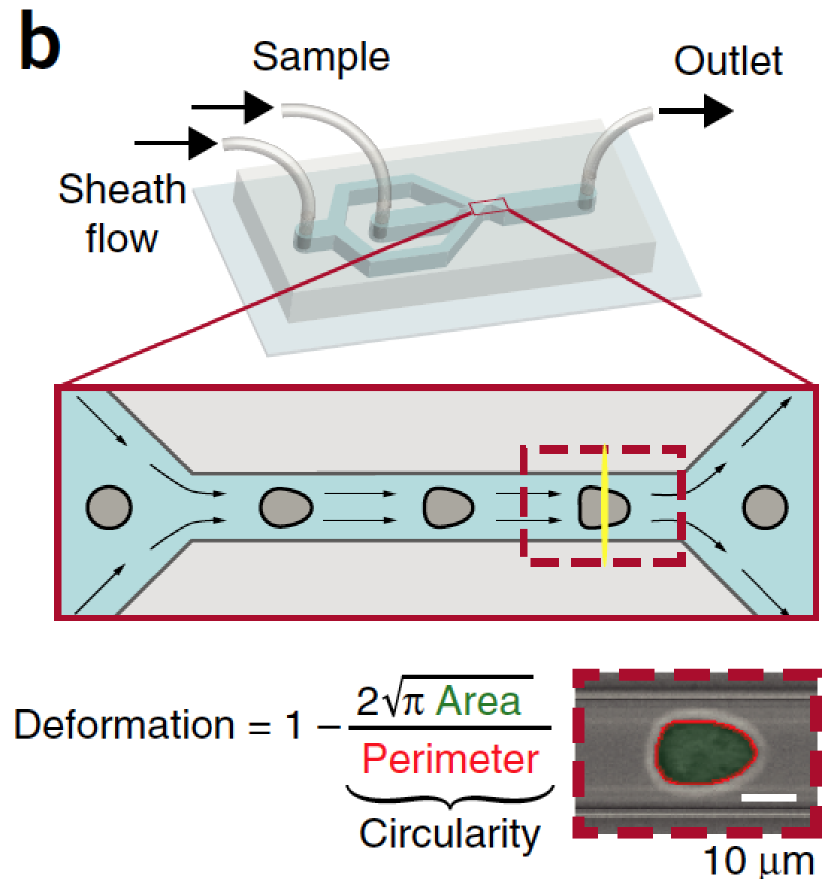
Real-time deformability cytometry

- Probing single cell deformability of >100,000 cells at 100 cells/s



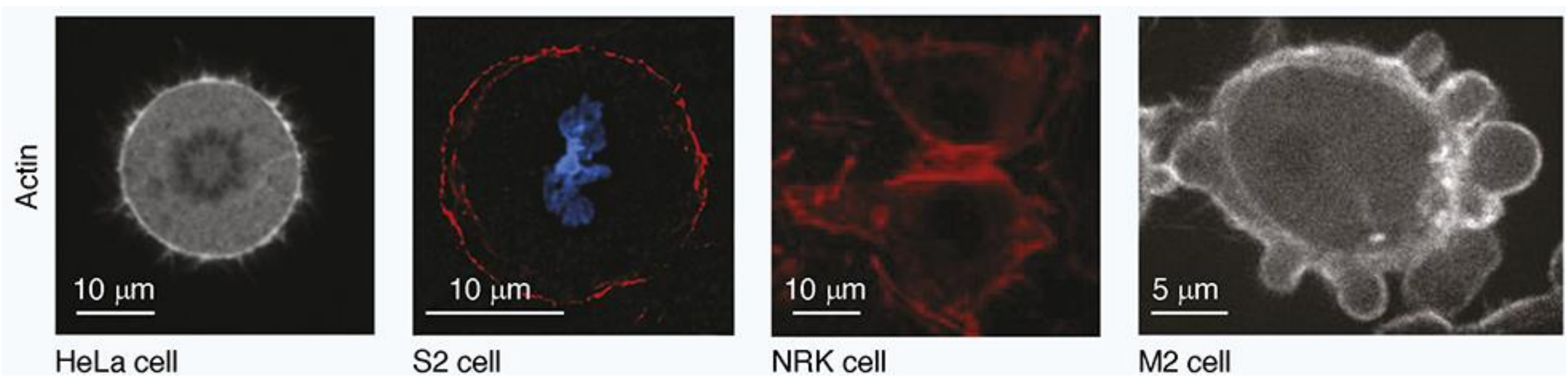
Real-time deformability cytometry

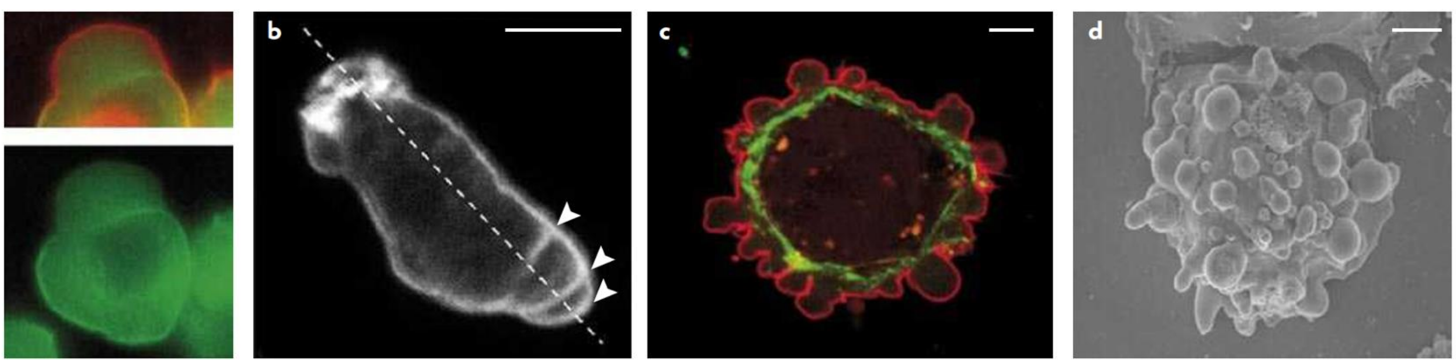
- 2,000 to 4,000 fps
- 1 μs LED pulses
- Flow rate: 10 cm/s
- Real-time measurement
    - Image processing
- Cell deformation is dependent on cell size
- Hydrodynamic model to calculate the shear stress and pressure
    - Decouples size and deformation
    - Isoelasticity lines with identical stiffness



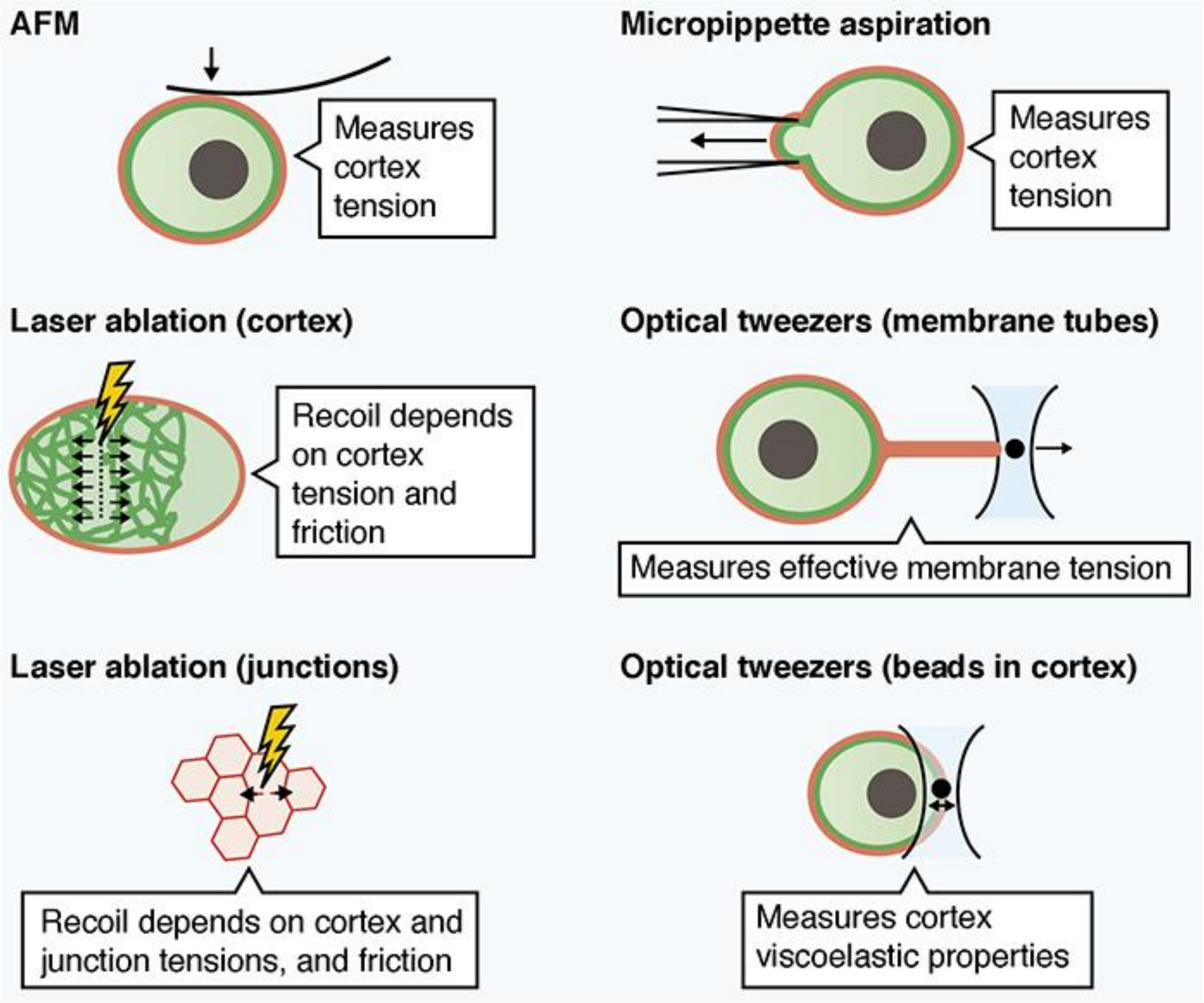
Actin Cortex

- Actin cortex is under tension
  - Myosin-driven contraction or crosslinkers
- Tension gradients
  - Migration, cell division, contact formation

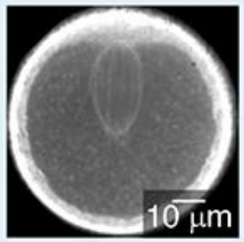
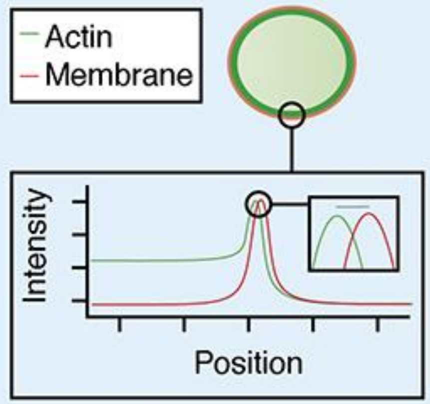
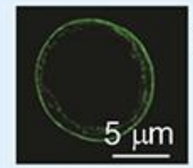
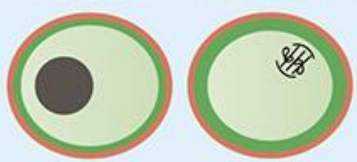




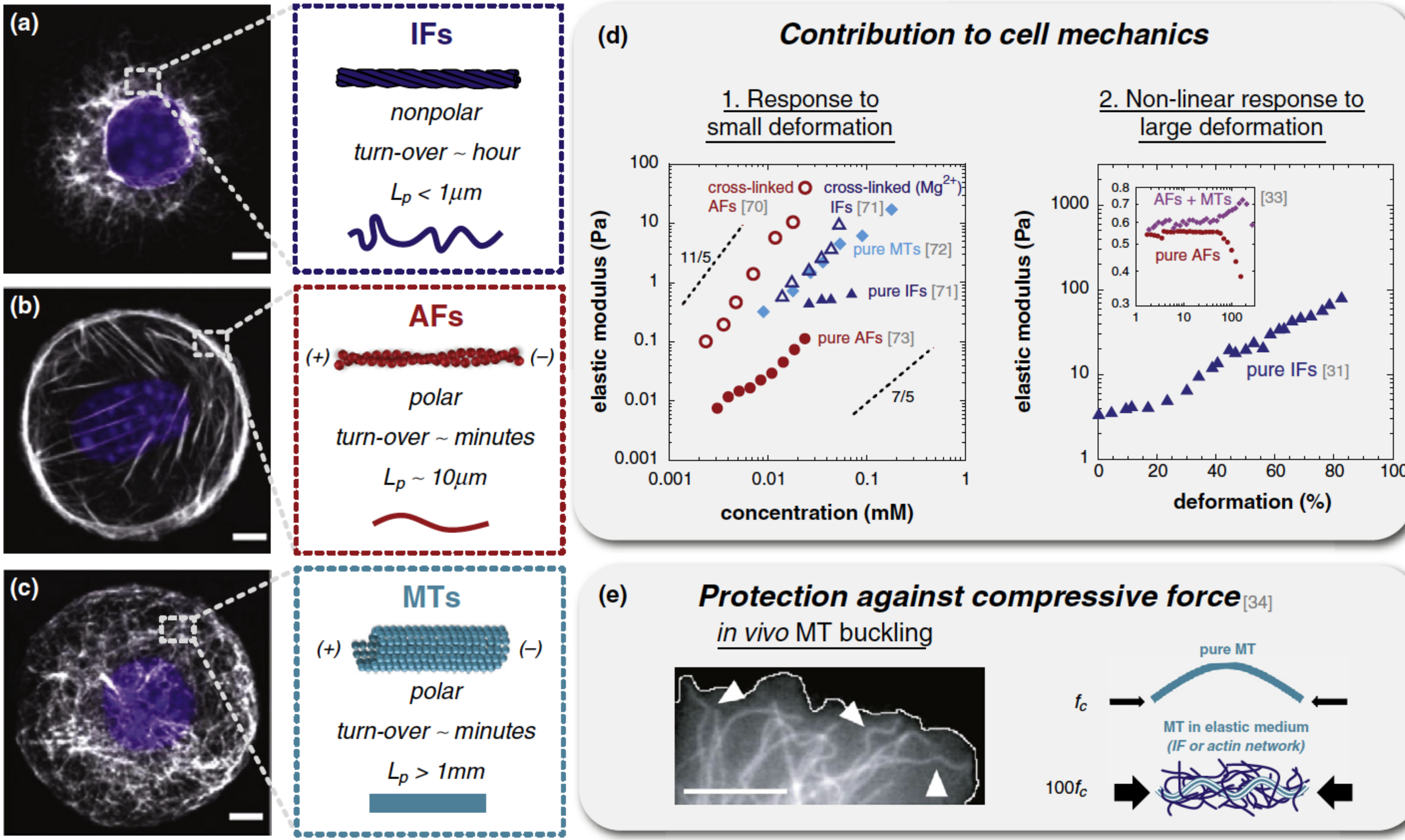
Measuring cortex mechanics



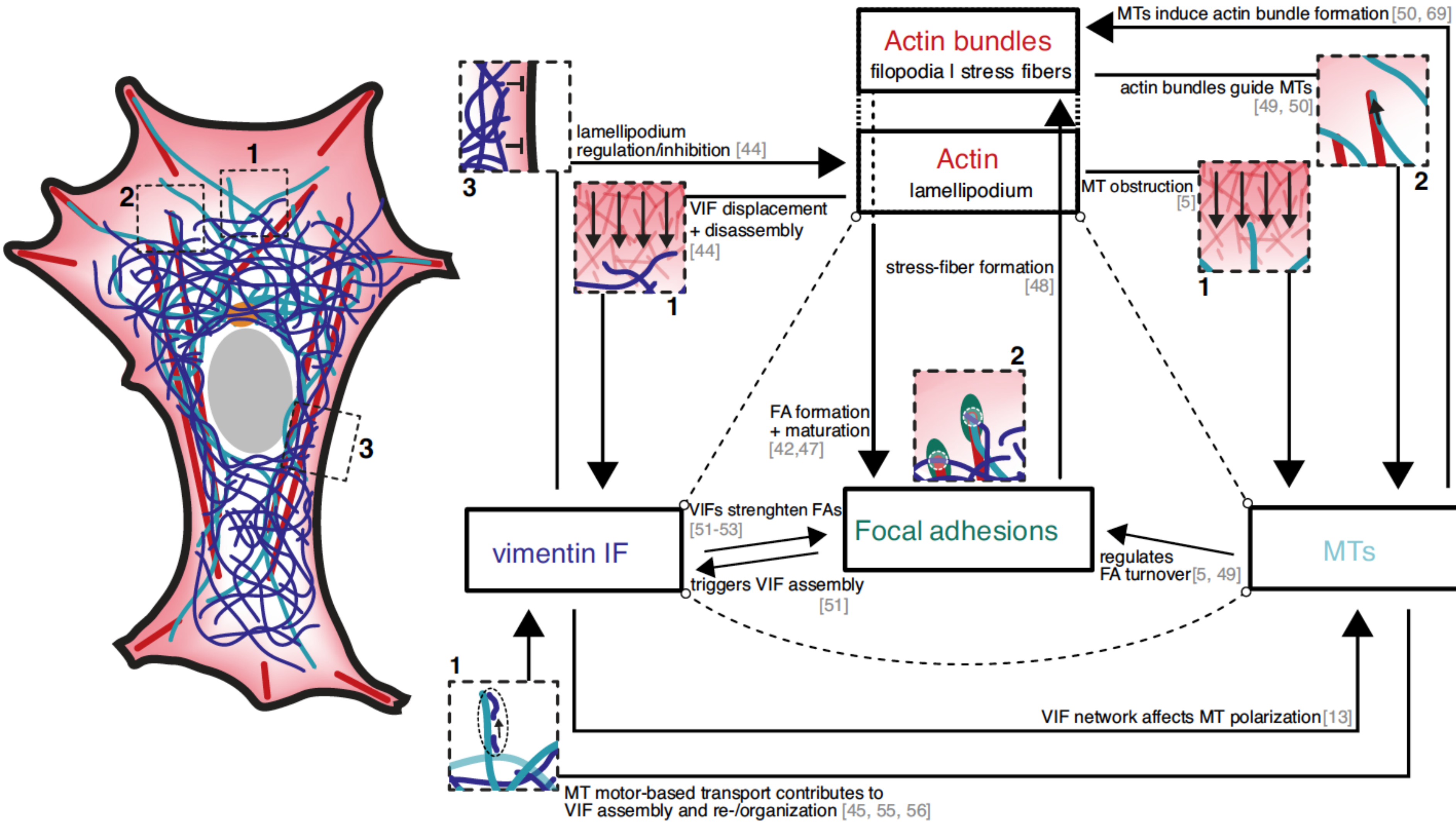
Cortex organization

	Thick cortices (oocytes)	Thin cortices	
Technique	Conventional light microscopy  (Image courtesy of A. Chaigne, J. Azoury, M.-E. Terret and M.-H. Verlhac) Mouse oocyte, meiosis I (F-actin labelled with Utrophin)	Subresolution image analysis 	STED microscopy  Jurkat T-cell (Clausen et al, 2017, *J. Physics D*, **50**, 6)
Measurement type	Direct	Indirect	Direct
Observations	 • Cortex thickness increases during meiosis-I • Arp2/3-dependent	 • Cortex thickness decreases between interphase and mitosis • Mitotic cortex thickness regulators: CAPZB, DIAPH1 and CFL1	

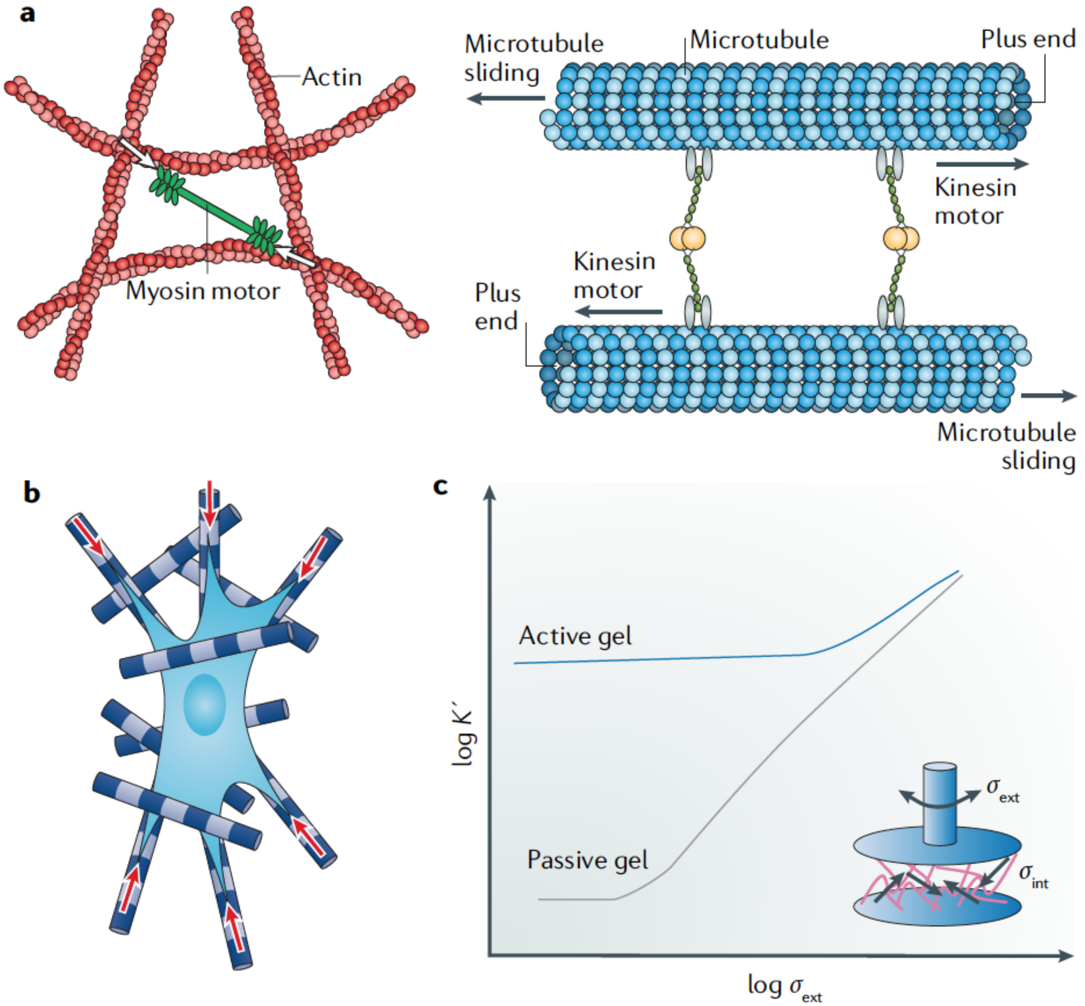
Cytoskeletal components and cell mechanics



Cytoskeletal crosstalk in migration

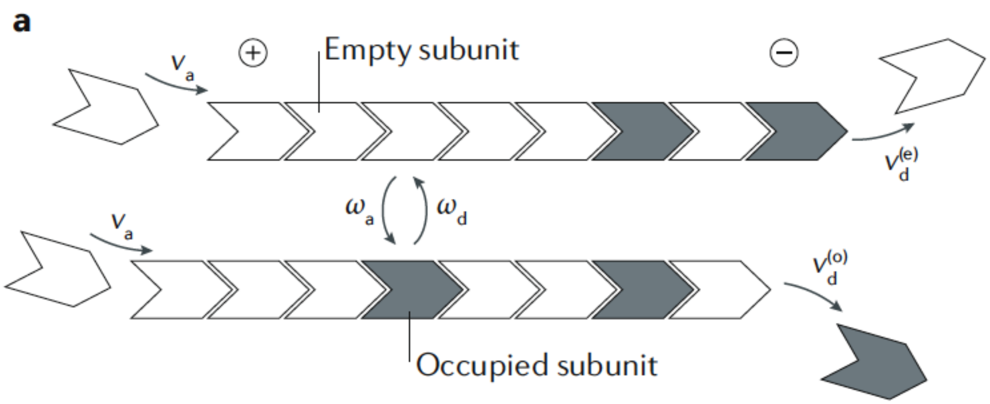


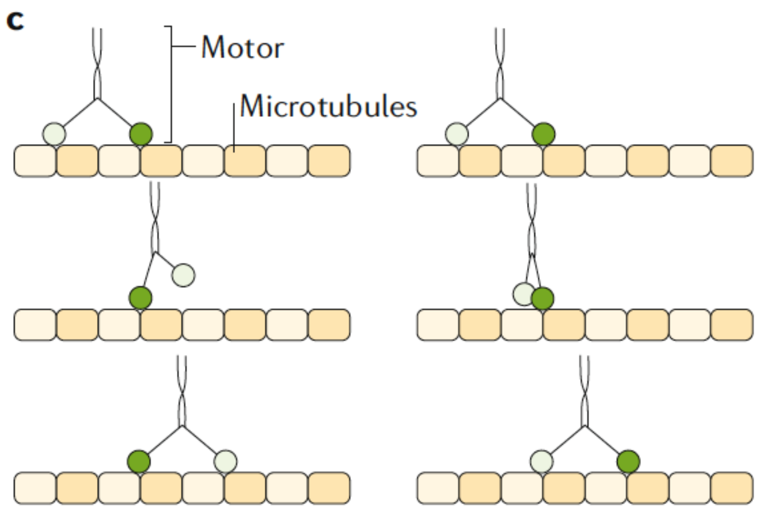
Active control over network mechanics



Models for cytoskeletal filaments and motors

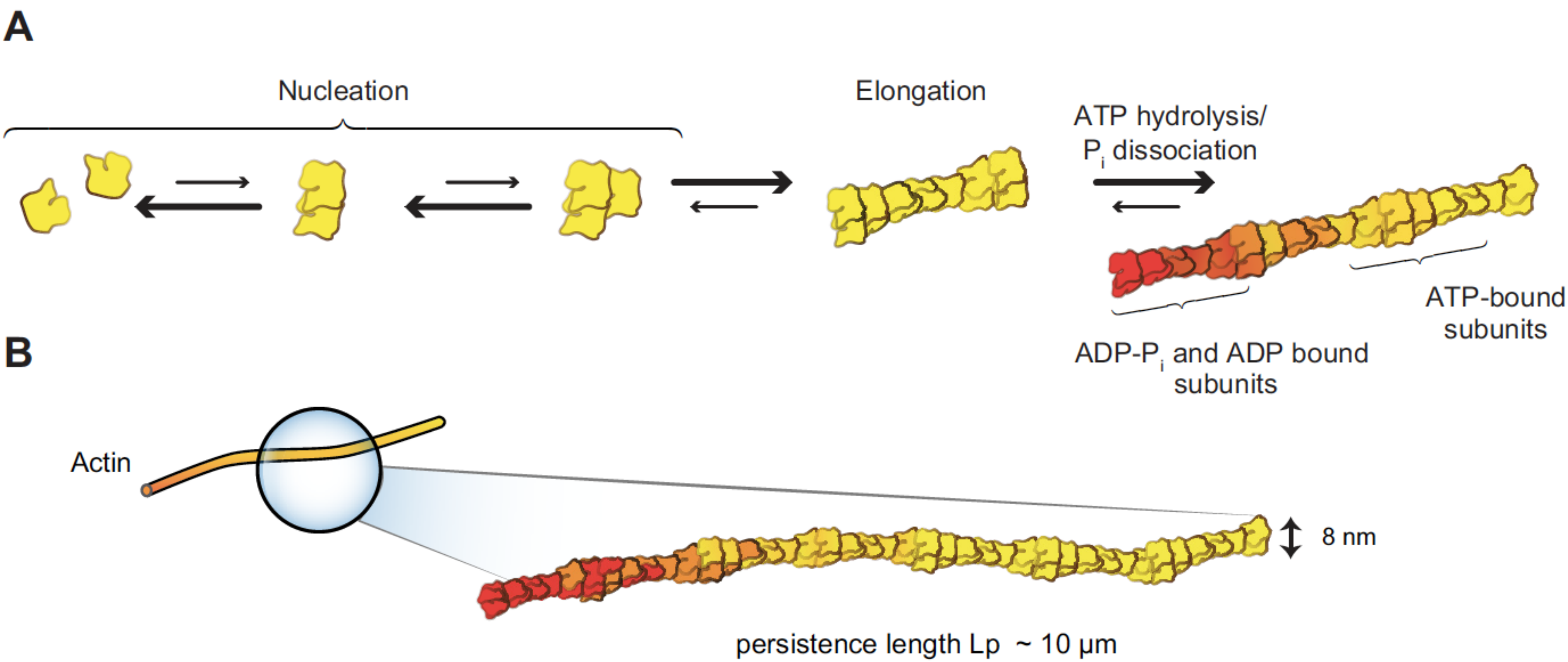
- Treadmilling filaments
- Thermal fluctuations and Brownian ratchet
- Polymerization and depolymerization rates
- Stepping of molecular motors
- Statistical mechanics and thermodynamics



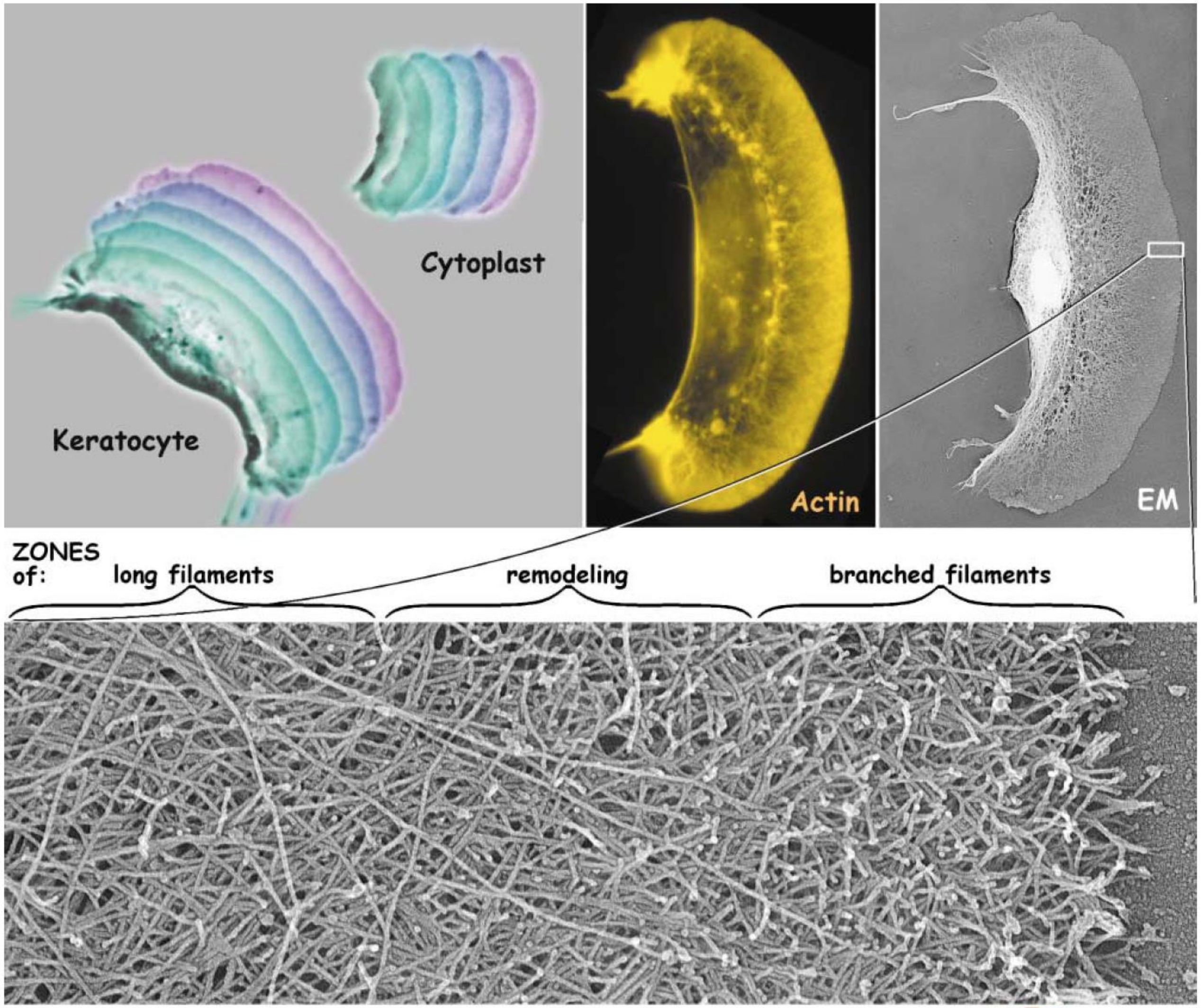


Actin assembly (movie)

- Monomeric globular form is the basic building block
- Nucleation: three actin monomers form a stable complex
- Formin facilitates elongation: 0.3 µm/min



Actin filament organization

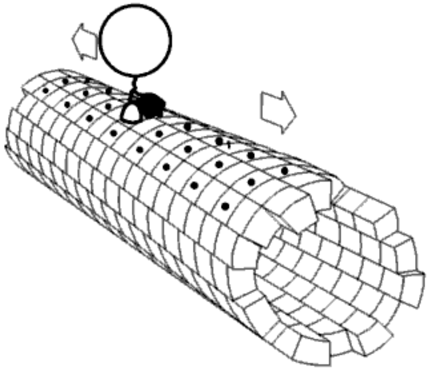


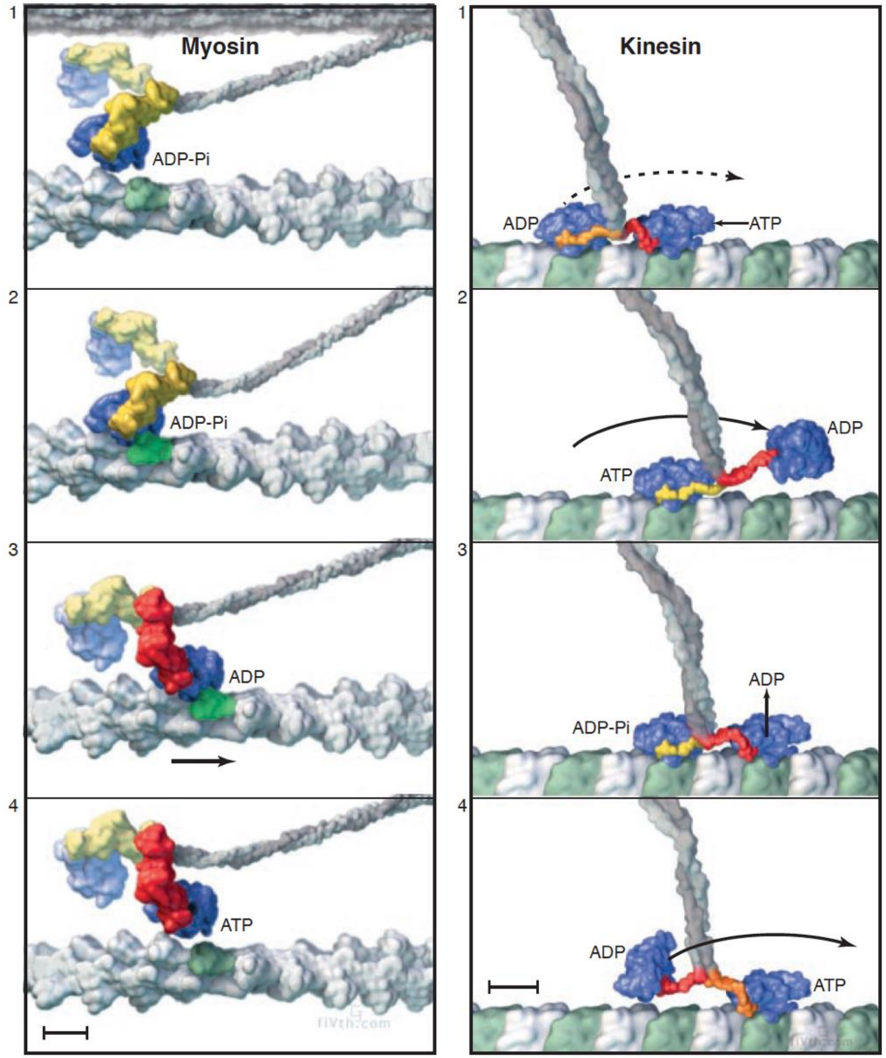
Motor proteins

- Motor proteins use the energy of ATP hydrolysis to move along microtubules or actin filaments.
  - **Myosin**: sliding of filaments relative to one another
  - **Kinesin/dynein**: transport of membrane-enclosed organelles along filament tracks
- The only structural element shared among all members of each superfamily is the motor “head” domain
- These heads can be attached to a wide variety of “tails,” which attach to different types of cargo and enable the various family members to perform different functions in the cell

- Highly ordered arrays of motor proteins that move on stabilized filament tracks
  - myosin-actin system of the sarcomere (muscle)
  - dynein-microtubule system of the axoneme (cilia and flagella)

Motor proteins (movie)



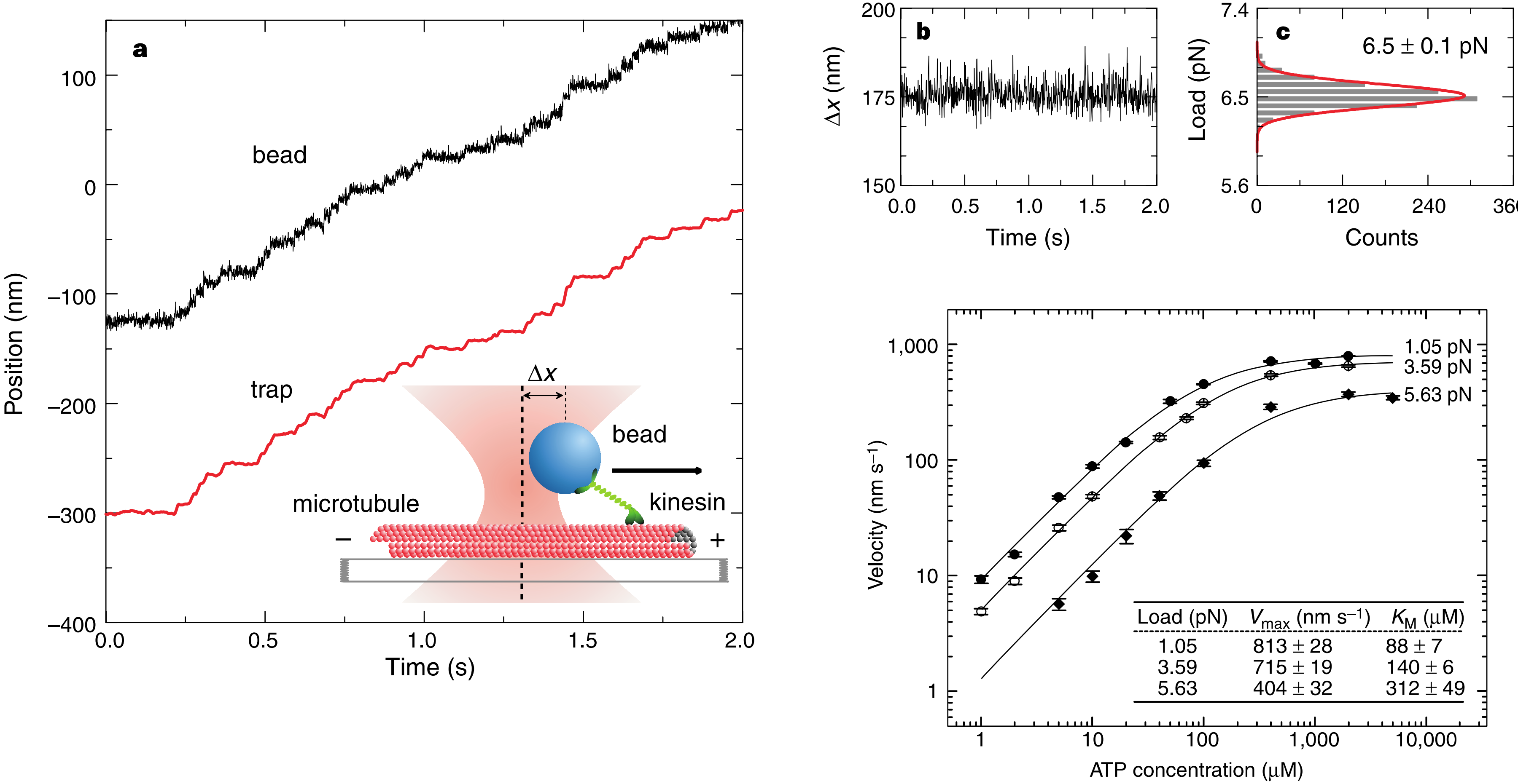


- Automobile
    - Breakdown of chemical fuel
    - Movement of piston
    - Crankshaft and transmission
    - Turning the wheels

- Piston like motion of relay helix
- Catalytic site, polymer binding site and mechanical element
- Conformational changes and ATP

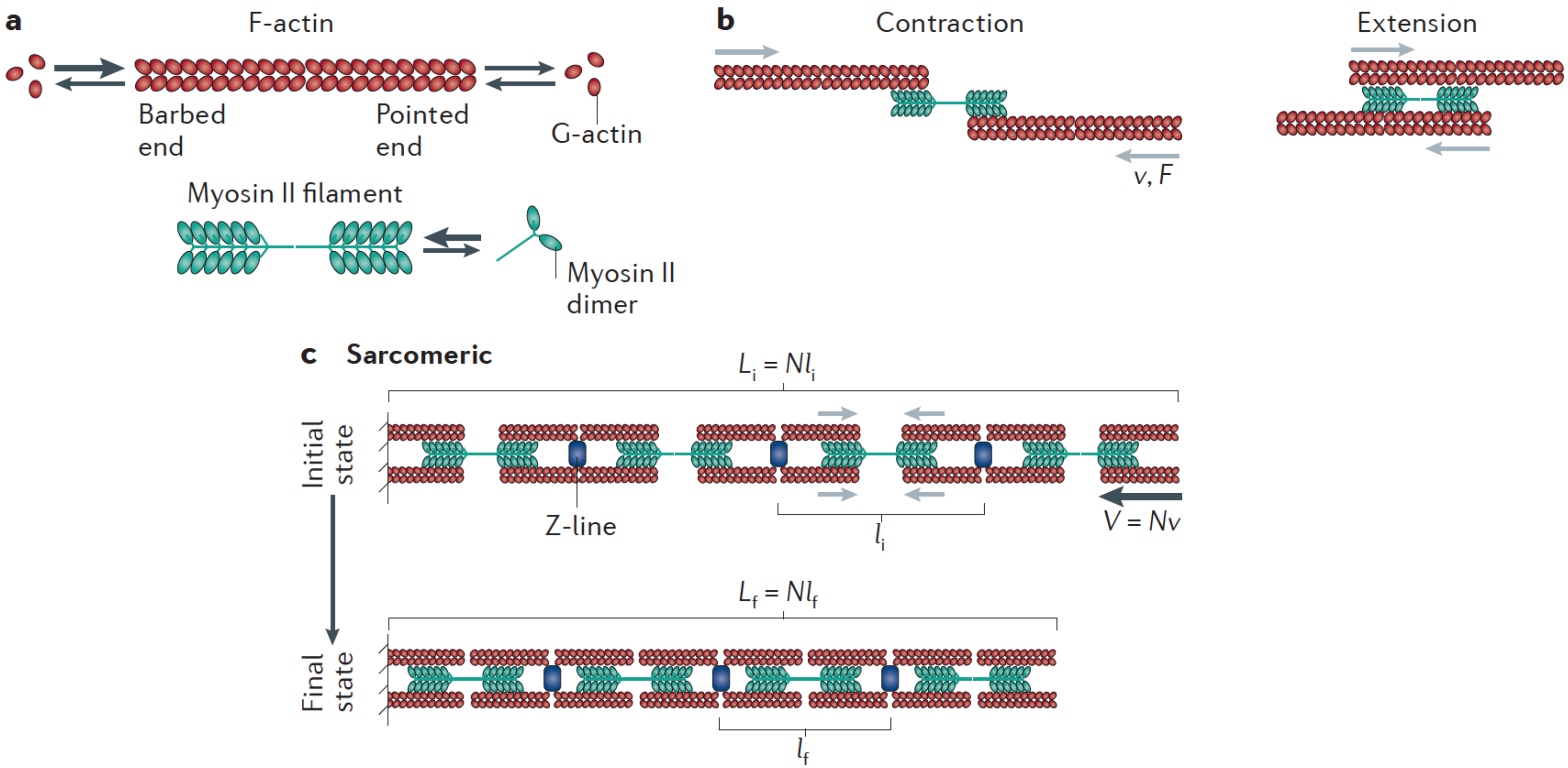
- Forward and reverse motors
- Processive vs non-processive
- Long step vs short step (8 vs 36 nm)

Motor proteins

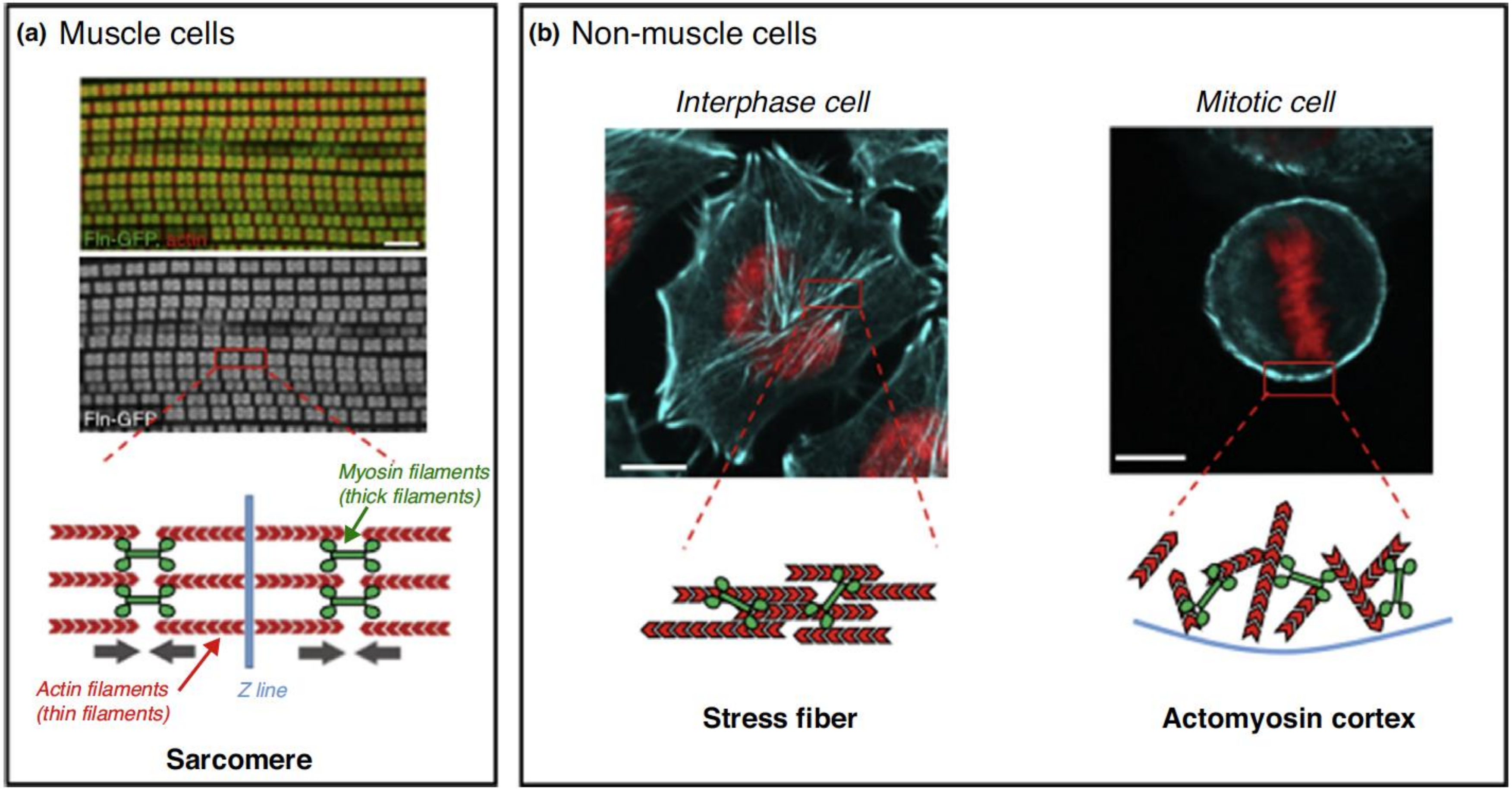


Load (pN)	V_{max} (nm s^{-1})	K_M (μM)
1.05	813 ± 28	88 ± 7
3.59	715 ± 19	140 ± 6
5.63	404 ± 32	312 ± 49

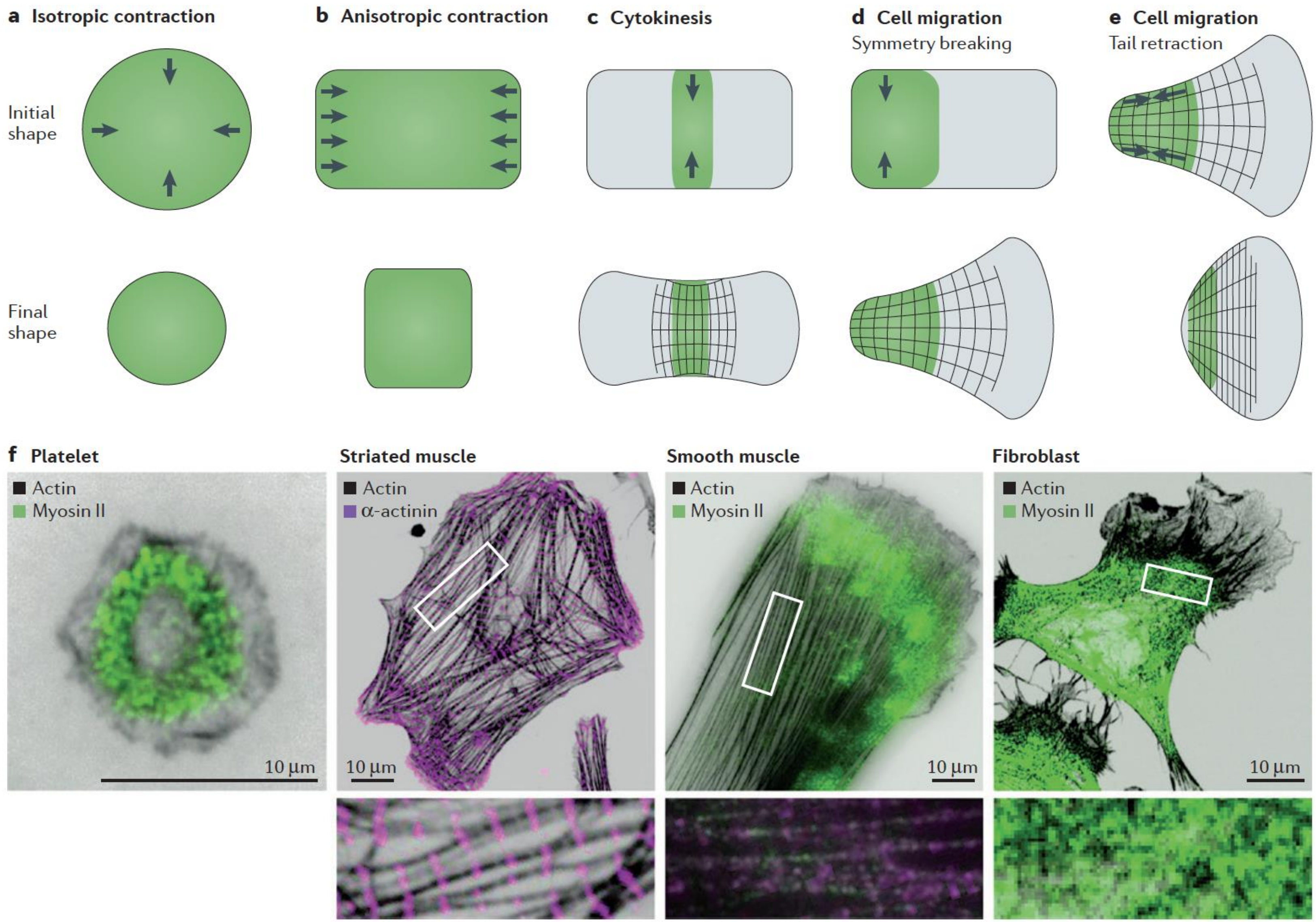
Actomyosin contractility



Muscle vs non-muscle cell structure



Contractile deformations



The nucleus

- Largest and stiffest organelle
- Nuclear envelope
  - Outer and inner nuclear membranes
  - Membrane-bound proteins and nuclear pore complexes
- Nuclear lamina
  - A filamentous protein network
  - Lamins and lamin-binding proteins
- Nuclear interior

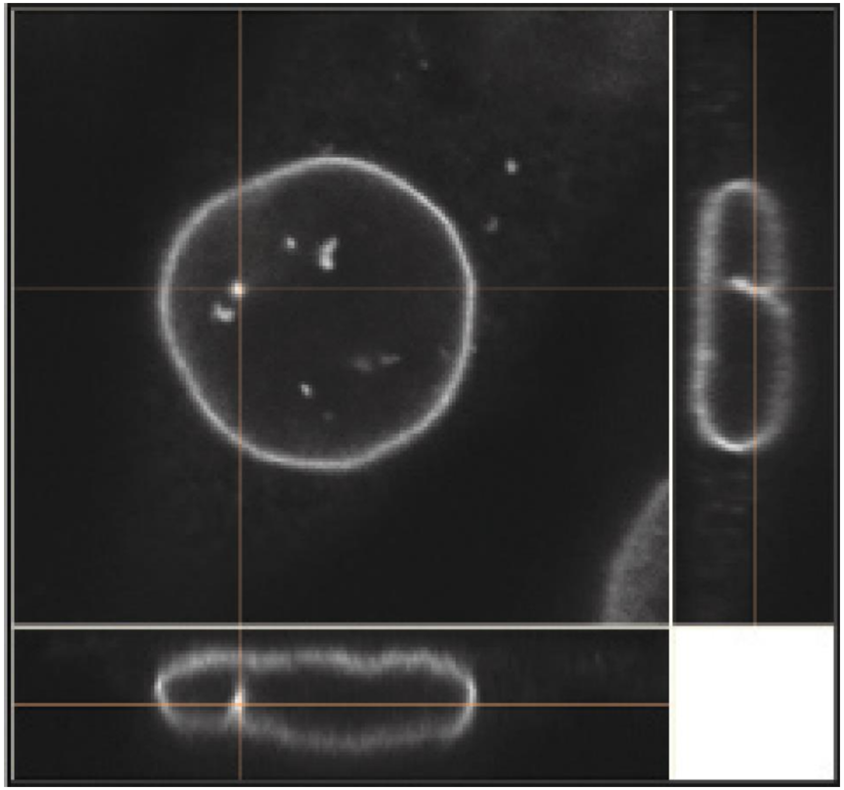
- Nuclear interior contains chromatin and nuclear bodies
- 4D nucleome: 3D chromatin architecture and its change over time
- Gene positioning
  - Peripheral localization promotes gene silencing
  - Interior positioning facilitates gene activation
- Chromatin condensation: epigenetic state
  - Nuclear deformability and internal pressure

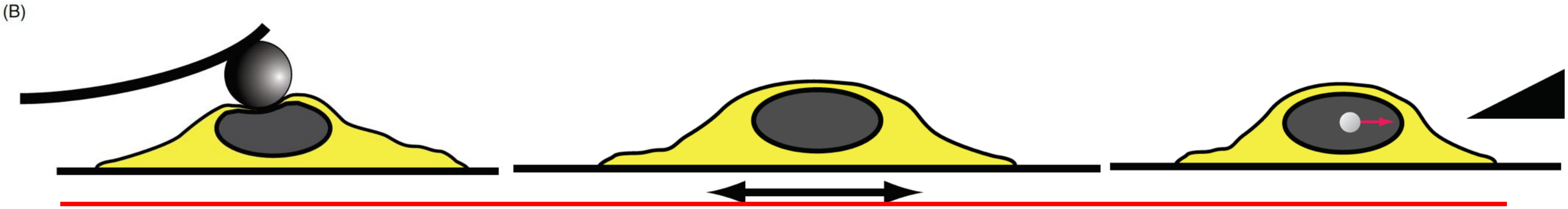
The nucleus

- 2D cell culture: flat disk
  - 10 - 20 µm diameter
  - 3 to 6 µm height
- In vivo spherical or ovoid
  - 6 to 10 µm diameter

- Nuclear stiffness: 1 to 10 kPa
- Cytoplasm: 0.5 kPa

- Physiologically relevant tissue strains (10 - 20%) correspond to 1 to 6 % nuclear elongation

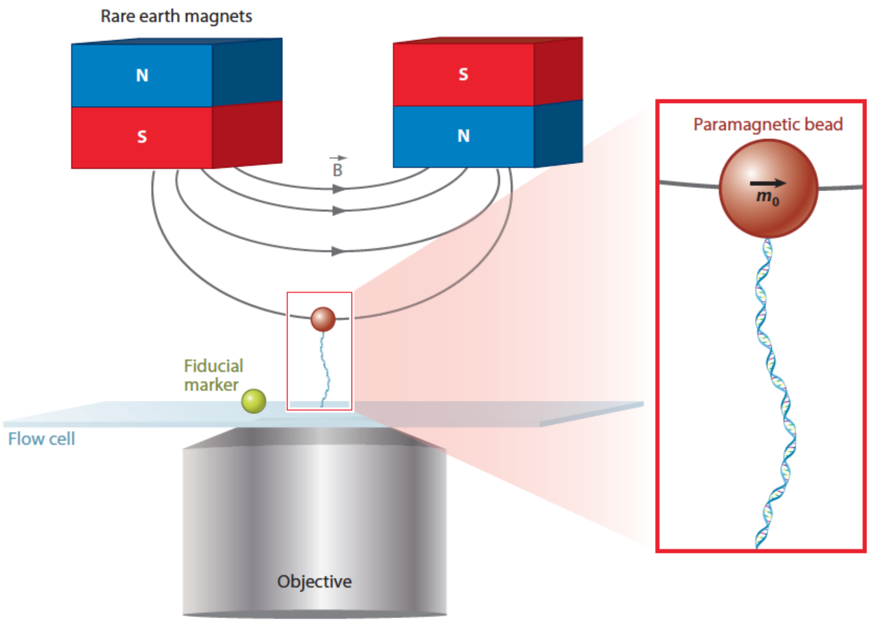


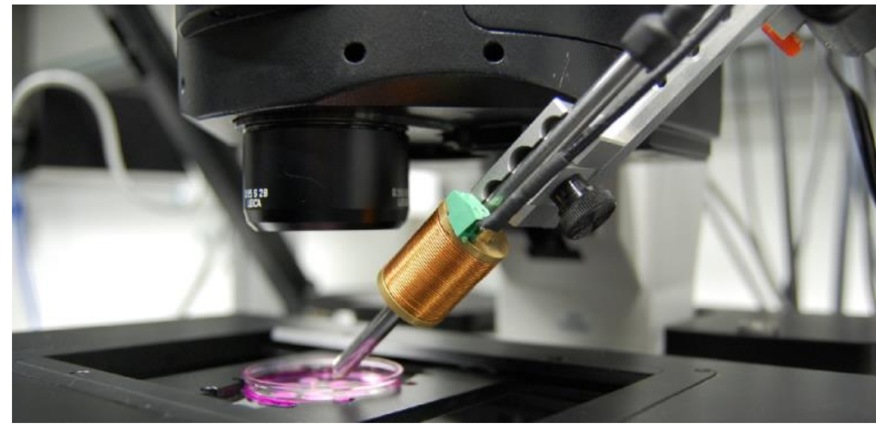


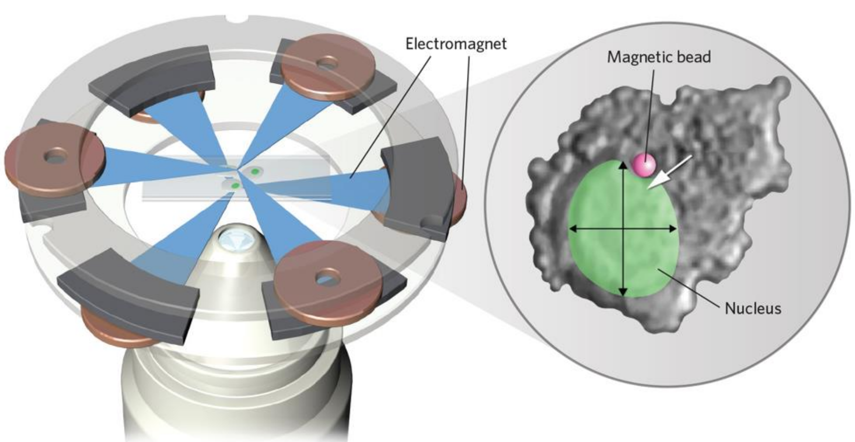
The nucleus

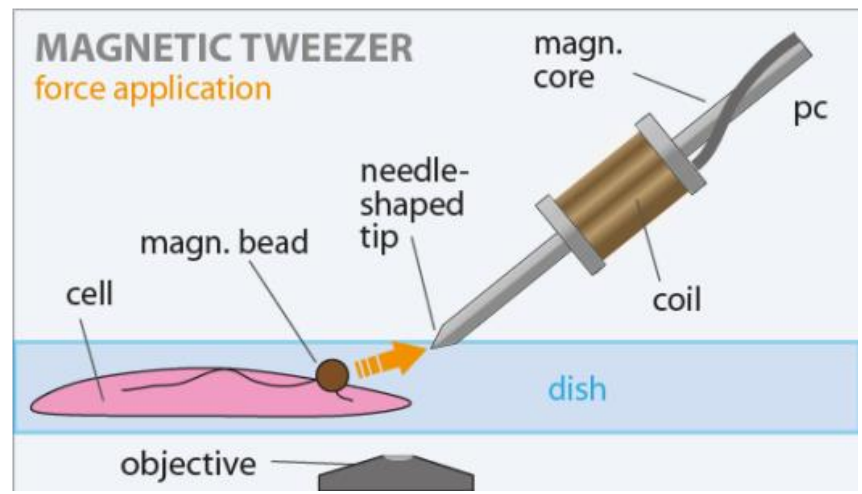
- Heterogenous structural organization → complex rheology
- Global response to micropipette aspiration
  - Viscoelastic: Two springs and a dashpot
  - Shifted to elastic load-bearing system in swollen nuclei
- Local response to magnetic pull force
  - Strain stiffening
- 3D chromatin cross-interactions: power-low rheology

Magnetic Tweezers

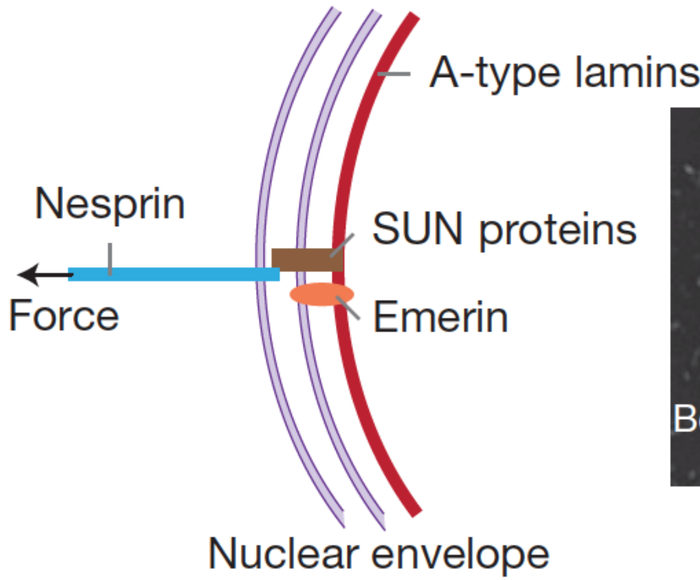


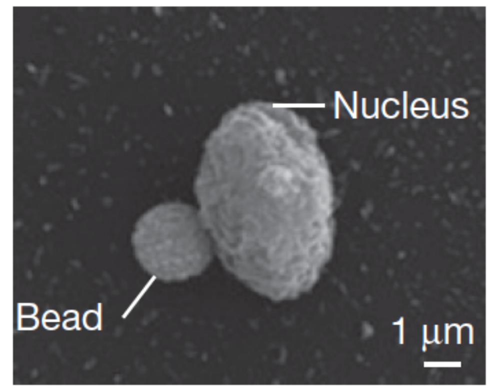




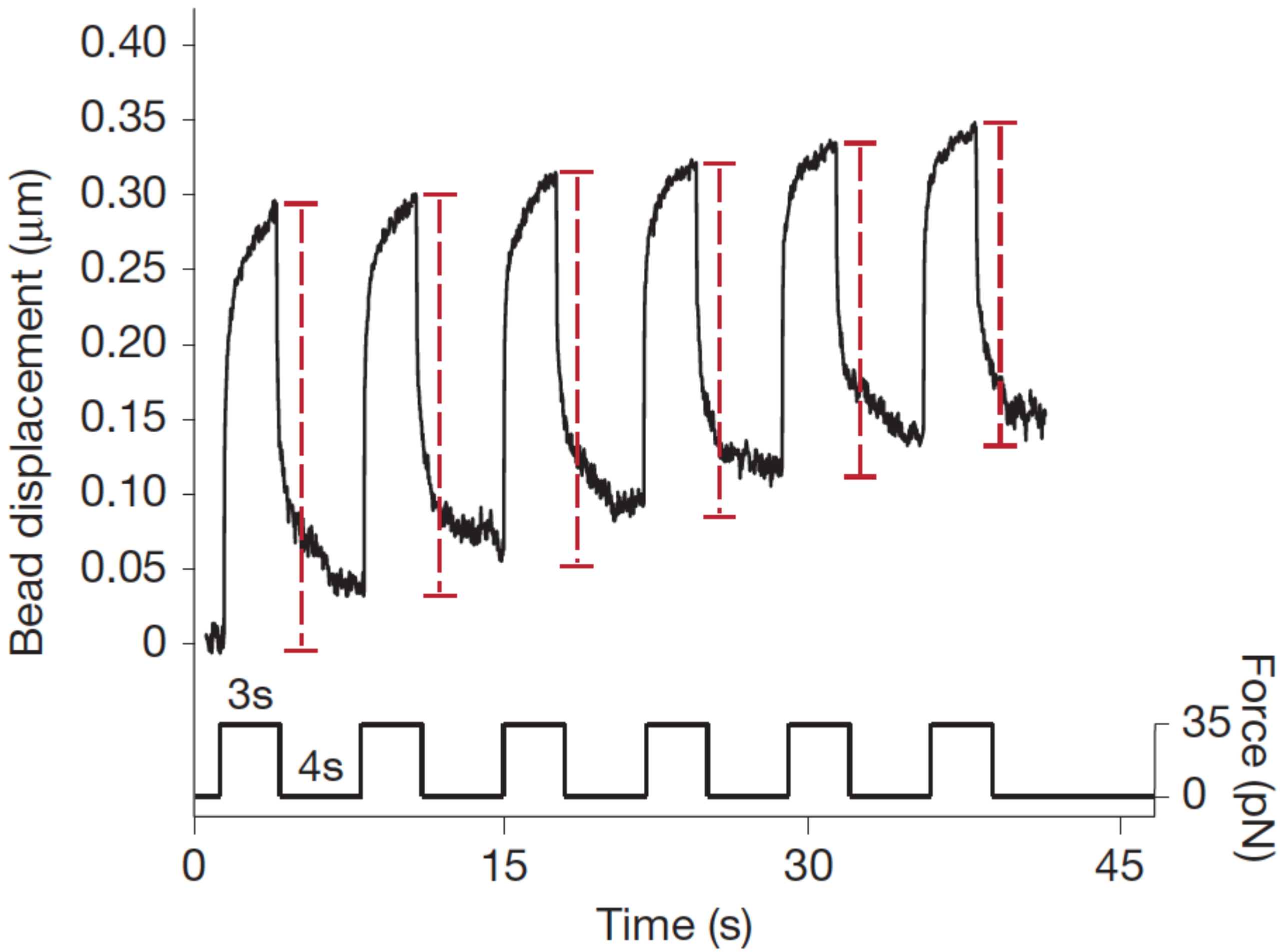


Isolated nuclei and strain stiffening



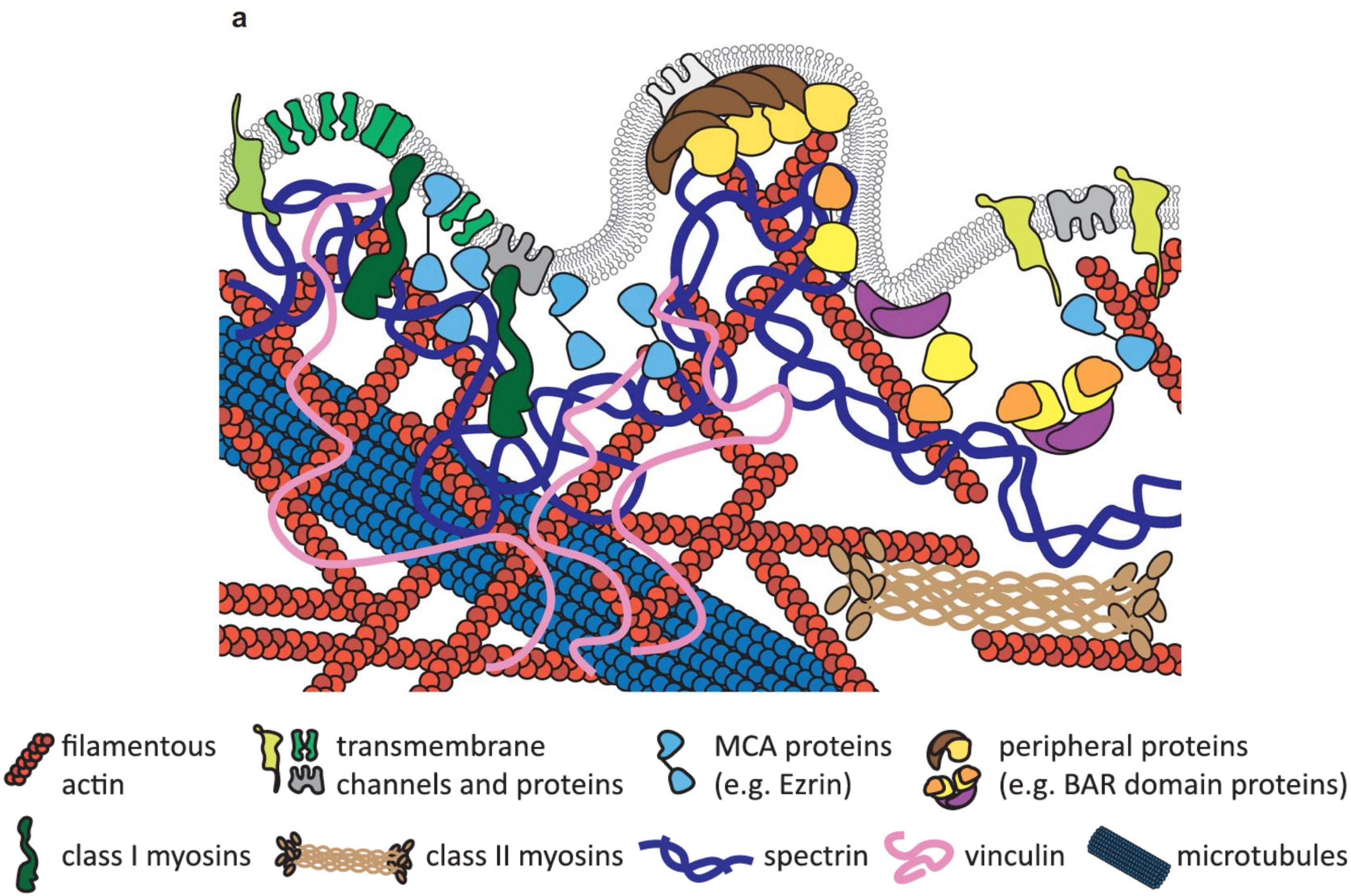


Isolated nuclei and strain stiffening



Plasma membrane

- Lipid bilayer with transmembrane and membrane bound proteins and sugars

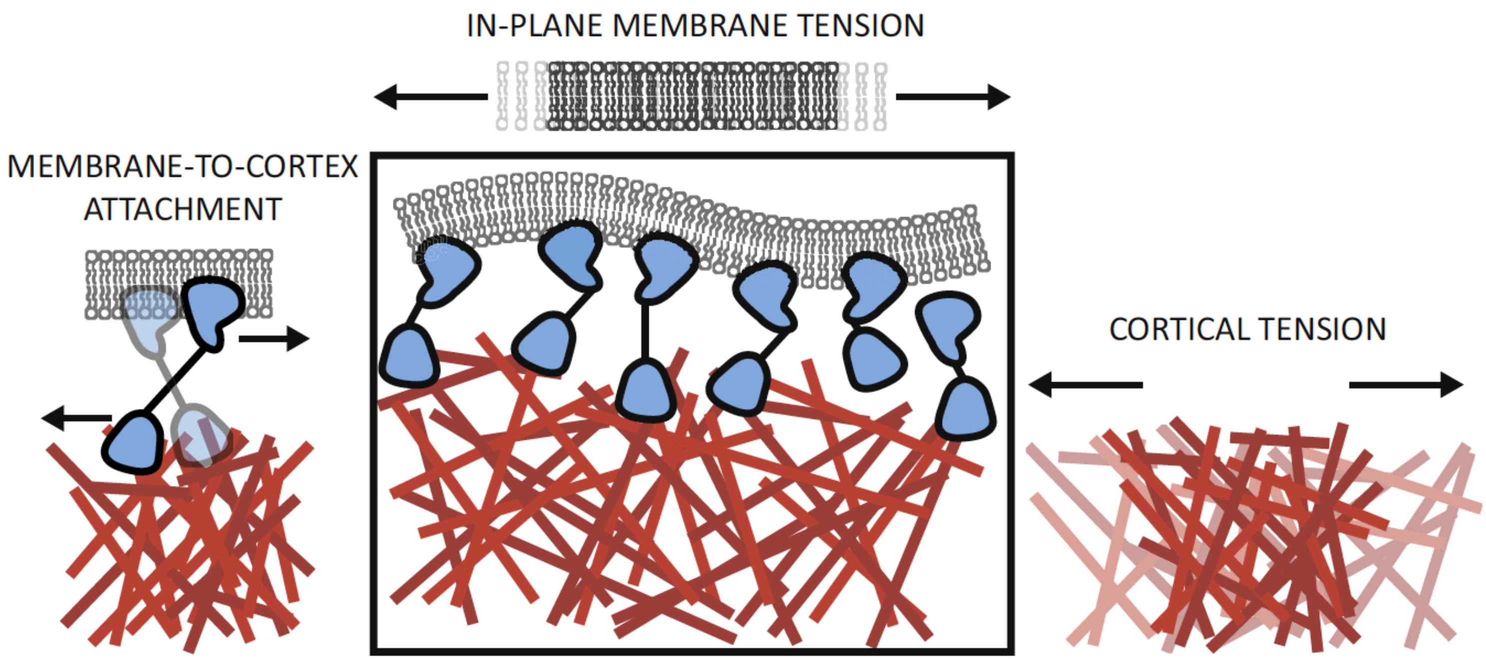


Membrane tension

- Membrane bending energy: $(k/2)\,C^2$
  - k is the bending stiffness and C is local curvature
- Bending stiffness is a few times the thermal energy ($20k_BT$, 80 pNnm)
- The interplay between membrane bending rigidity and membrane tension defines membrane shape
- Cellular membrane tensions tend to be on the order of 100 $pN\mu m^{-1}$, so in order to pull membrane tethers, forces in the range of 25 pN must be applied

Membrane tension

- Hard to distinguish between membrane tension and cortical tension
  - Pharmacological agents

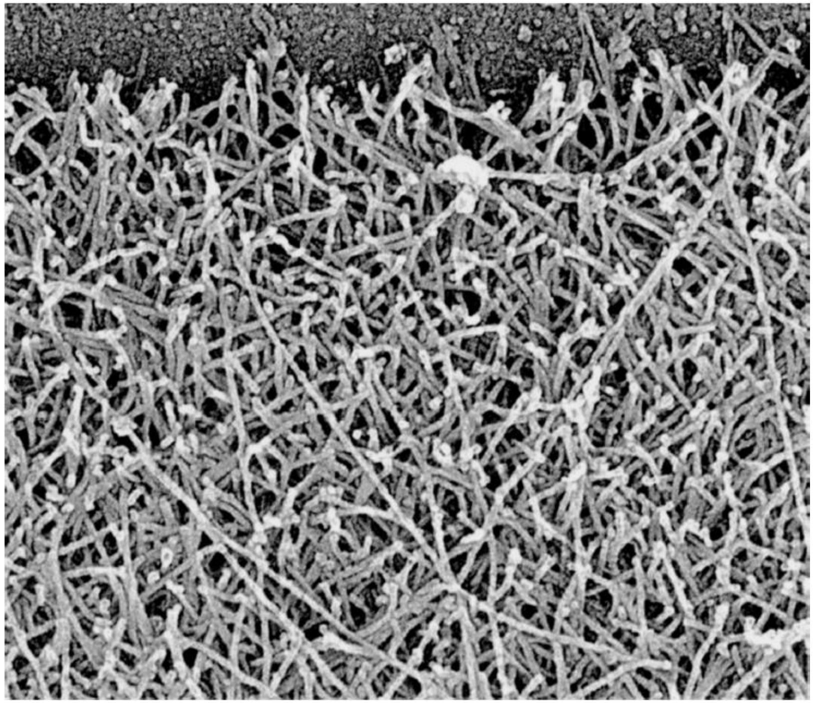


Why do animal cells move?

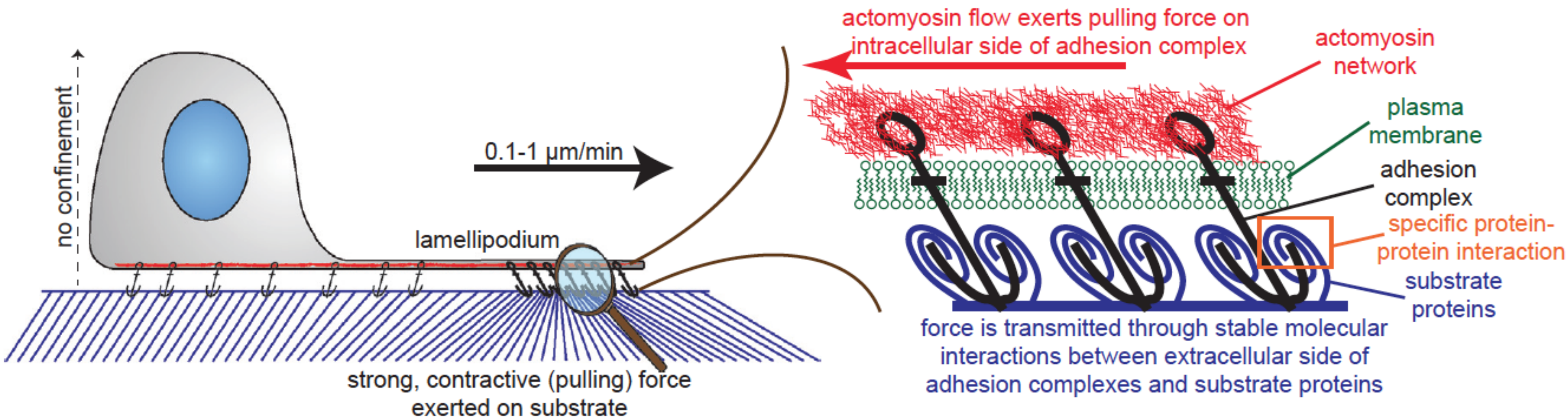
- Morphogenic movements during embryonic development
- Movement of neurites
- Immune cells
- Fibroblasts and wound healing

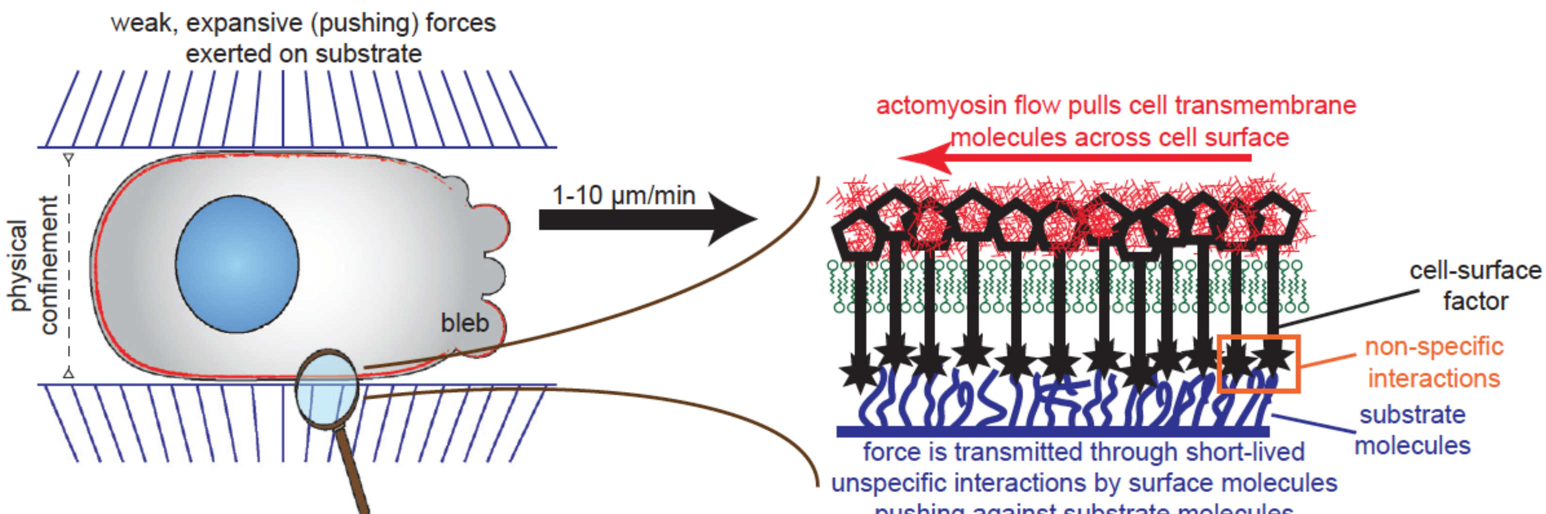
Cell migration

- Pseudopods: lamellipodia, filopodia, lobopodia
- Best studied examples
  - The leading edges of the fish and amphibian keratocytes
  - Large and extremely thin lamellipodia at the edge



Adhesive vs non-adhesive migration



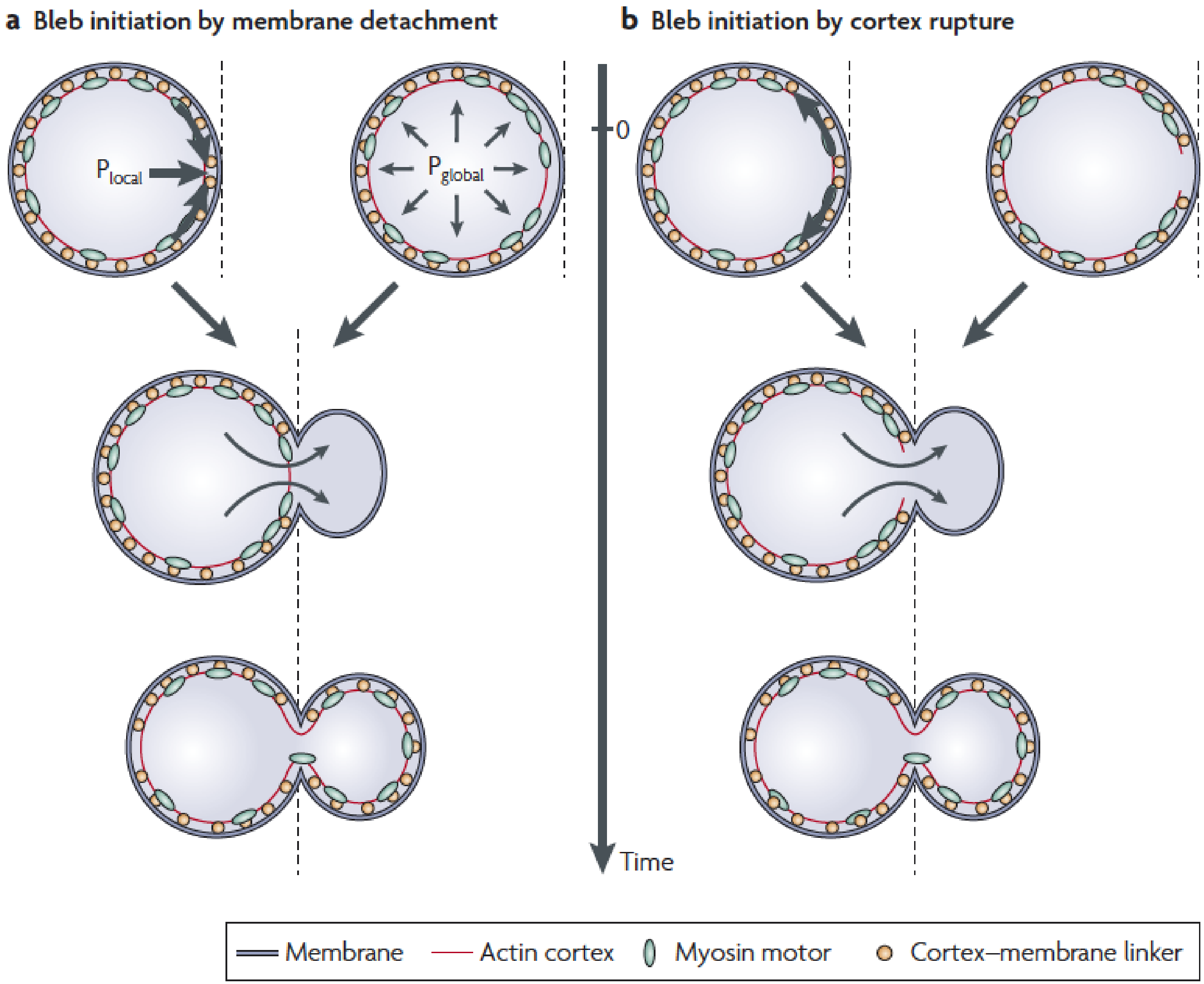


Bleb

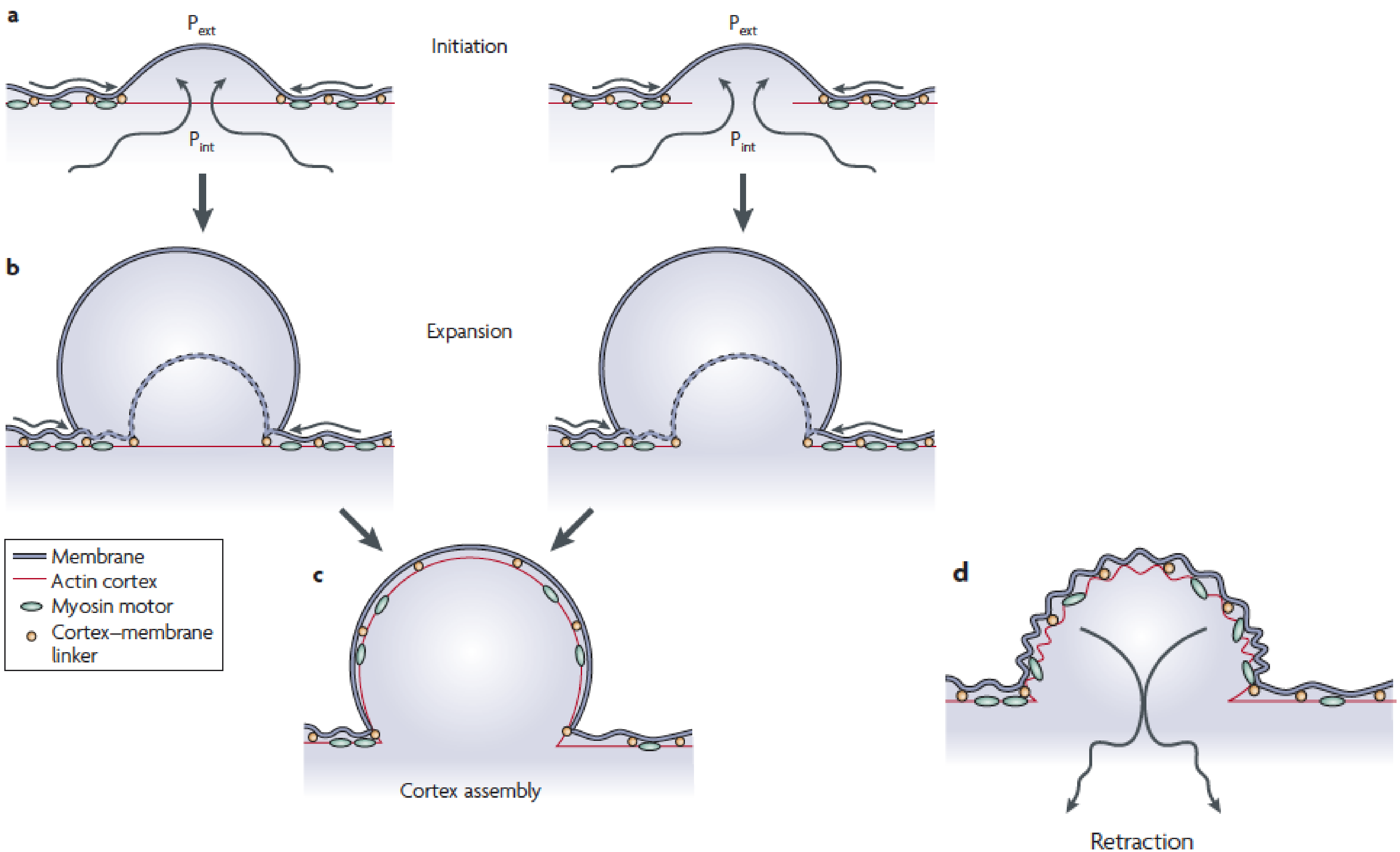
- A protrusion of the plasma membrane
  - Transient detachment of the membrane from the actin cortex
  - Rupture in the actin cortex

- Cytosol streams out of the cell body and inflates the newly formed bleb
  - Lasts 5 to 30 s
- Actin cortex is reconstituted
  - Lasts 60 to 120 s

Bleb


a Bleb initiation by membrane detachment
b Bleb initiation by cortex rupture
P_{local}
P_{global}
0
Time
Membrane
Actin cortex
Myosin motor
Cortex–membrane linker

Bleb lifecycle

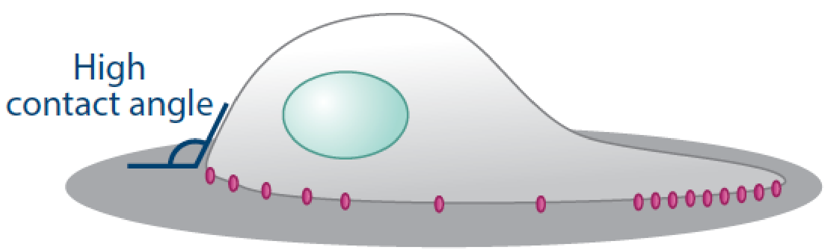


Adhesive vs non-adhesive migration

a Mesenchymal migration

Key molecules:
talin, integrin, actin, and
other well-studied molecules

Cell velocity
slow: ~0.1–0.5 μm/min



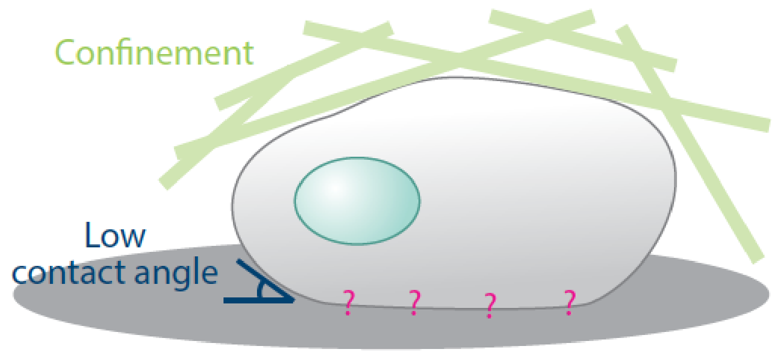
Well-studied substrate adhesion

Commonly used by fibroblasts, keratinocytes, endothelial cells, and cancer cells

b Amoeboid migration

Key molecules:
actin, myosin, and unidentified
force-generating molecules

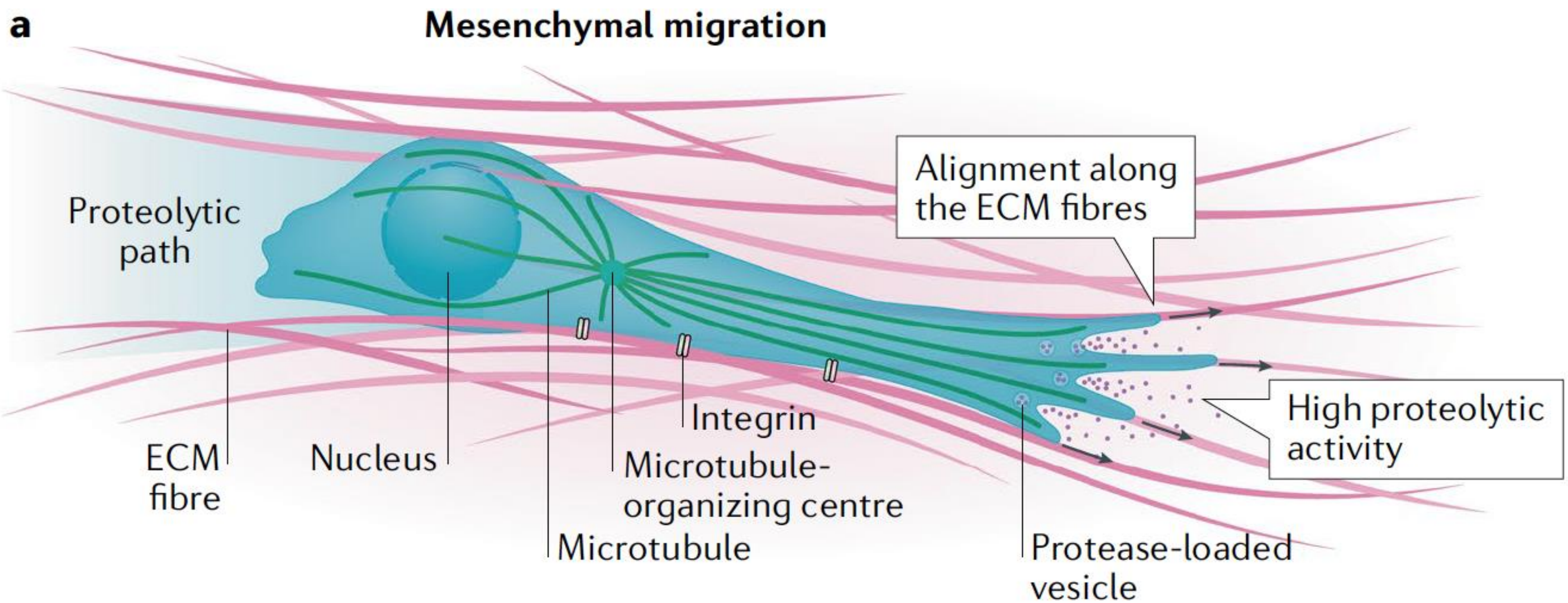
Cell velocity
fast: ~5–20 μm/min



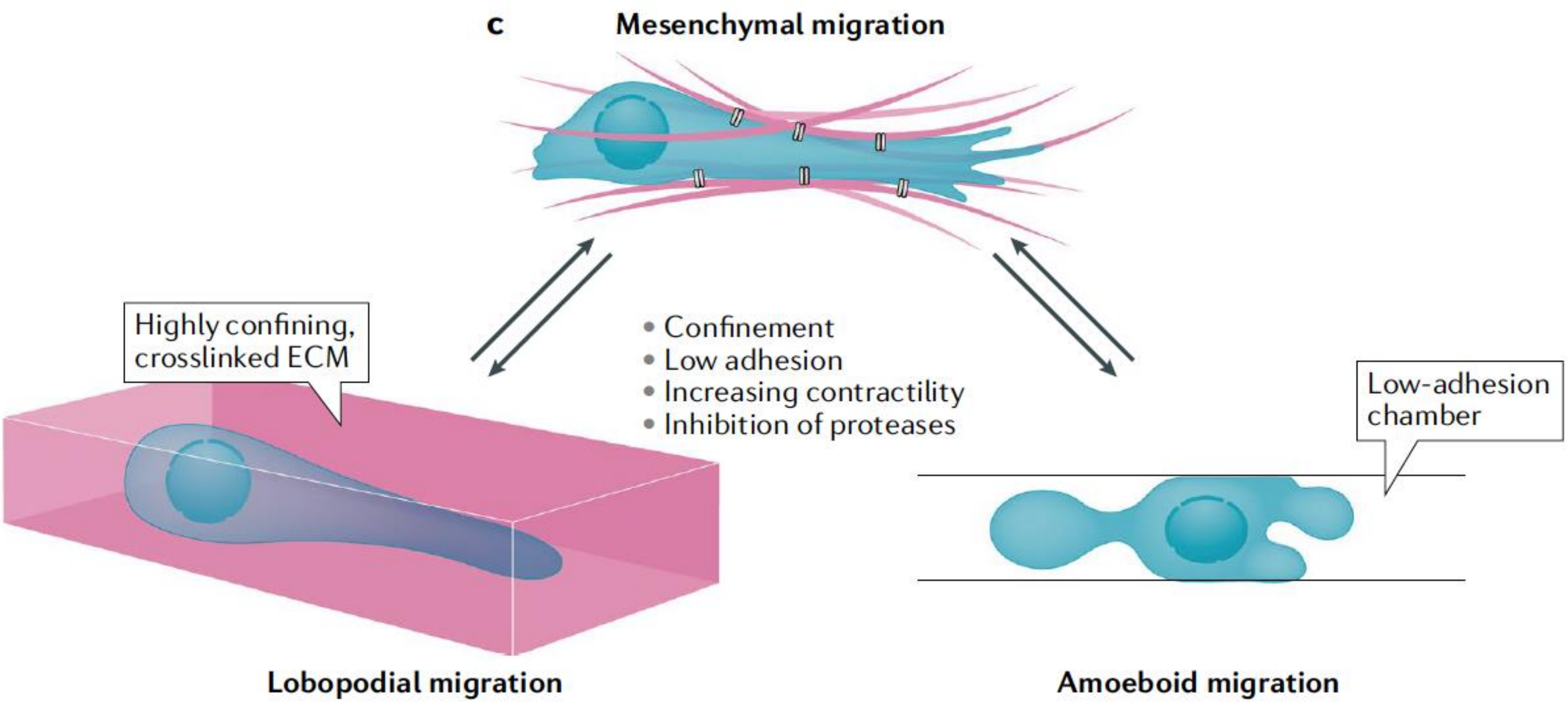
Poorly understood substrate interaction

Commonly used by cells of the hematopoietic lineage (lymphocytes, dendritic cells, T cells), cancer cells, and cells in developing embryos

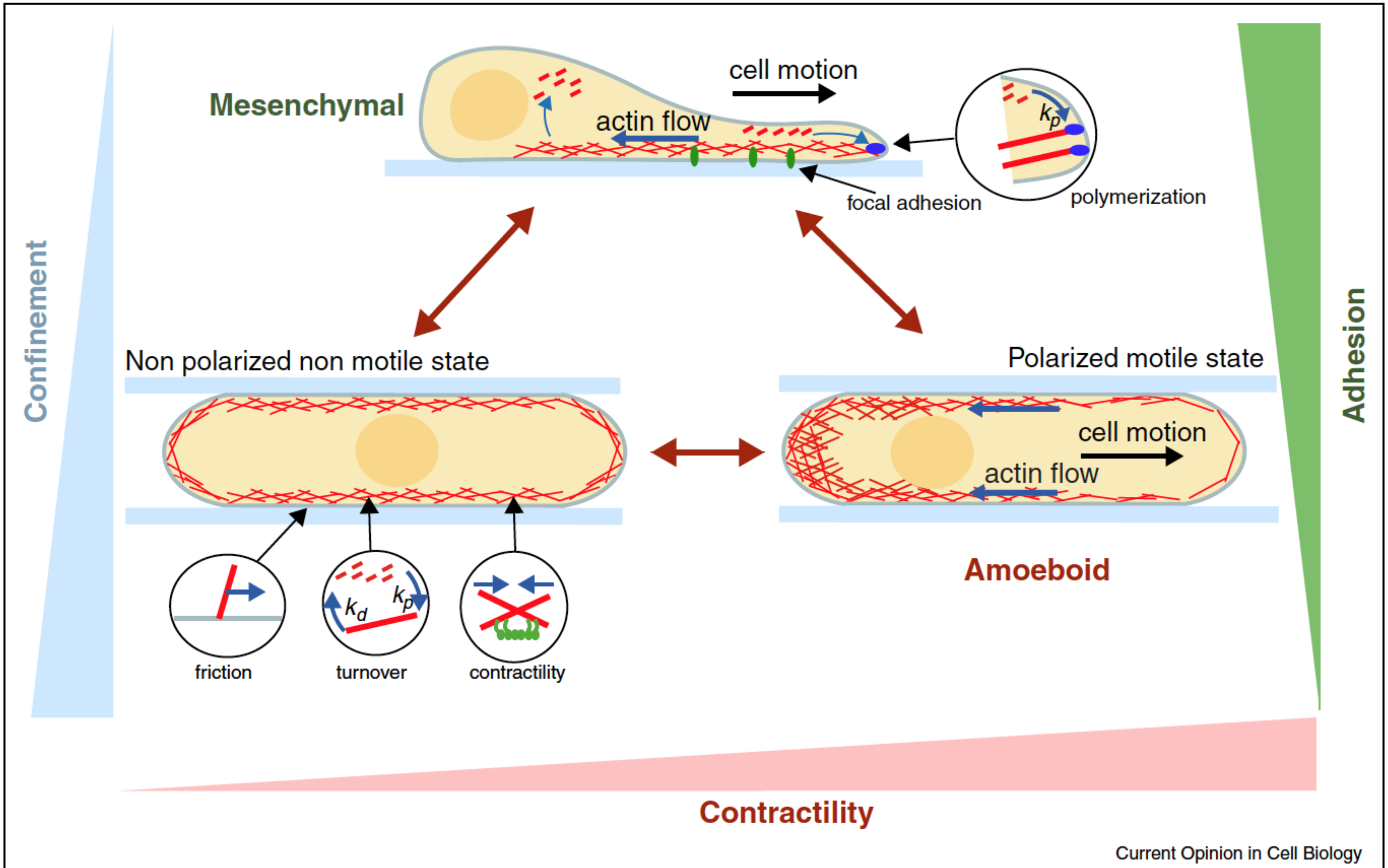
Mesenchymal migration



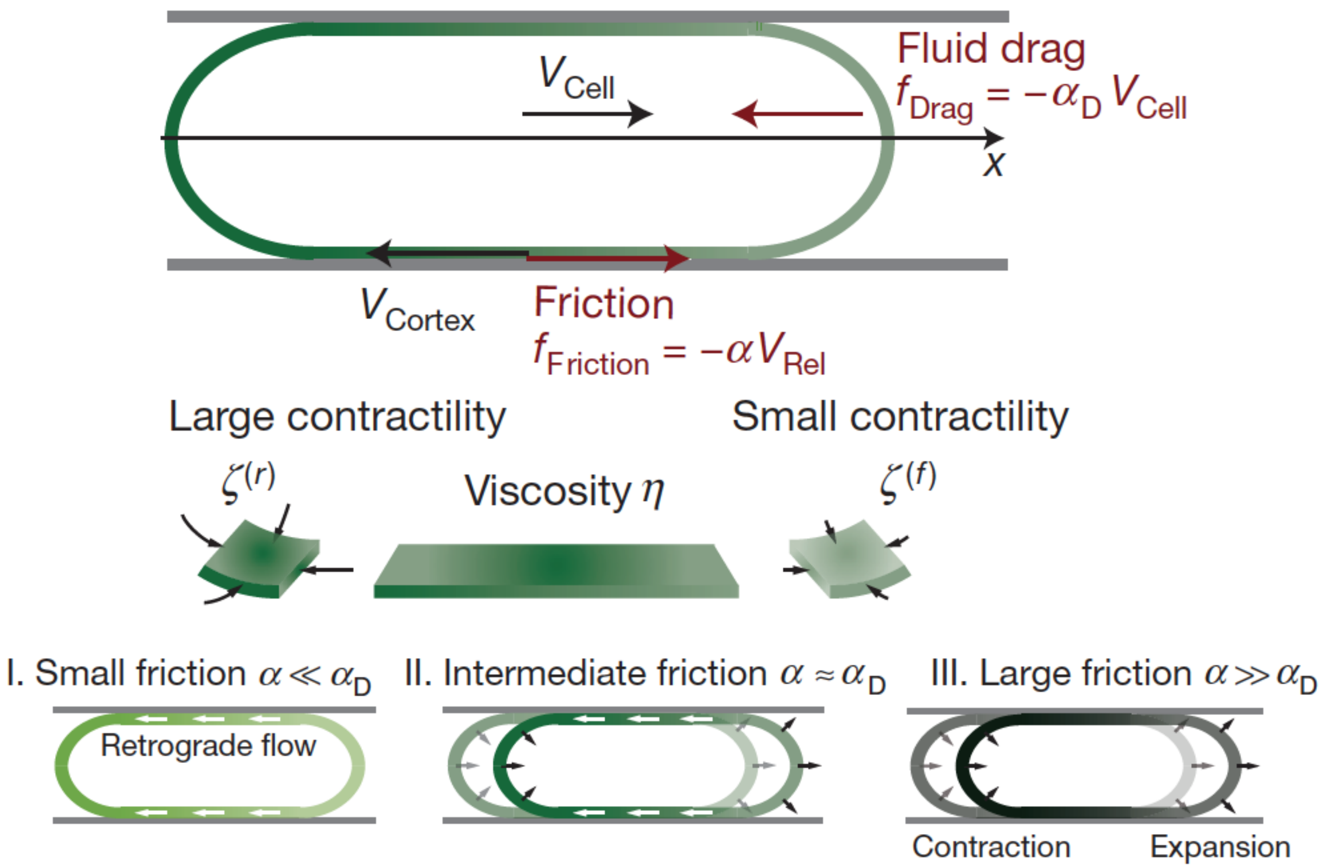
Adaptive features and plasticity



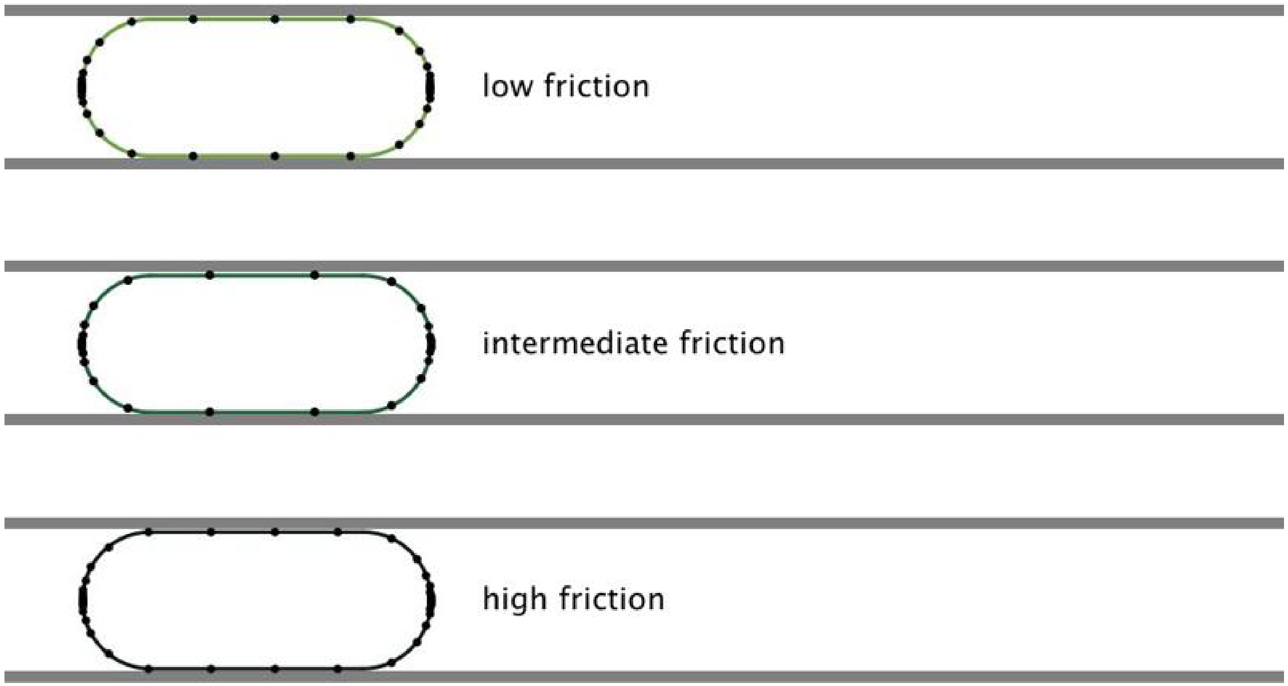
Adaptive features and plasticity

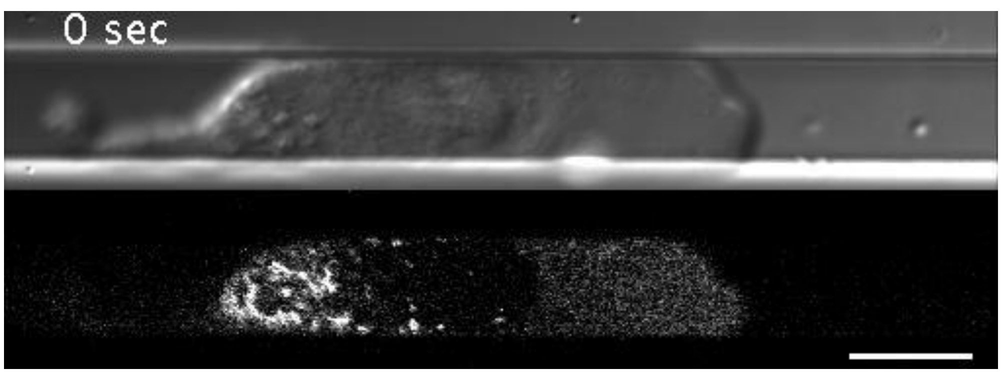


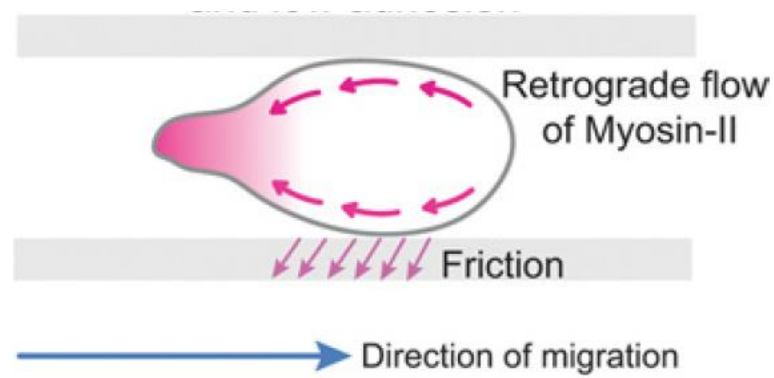
Retrograde flow



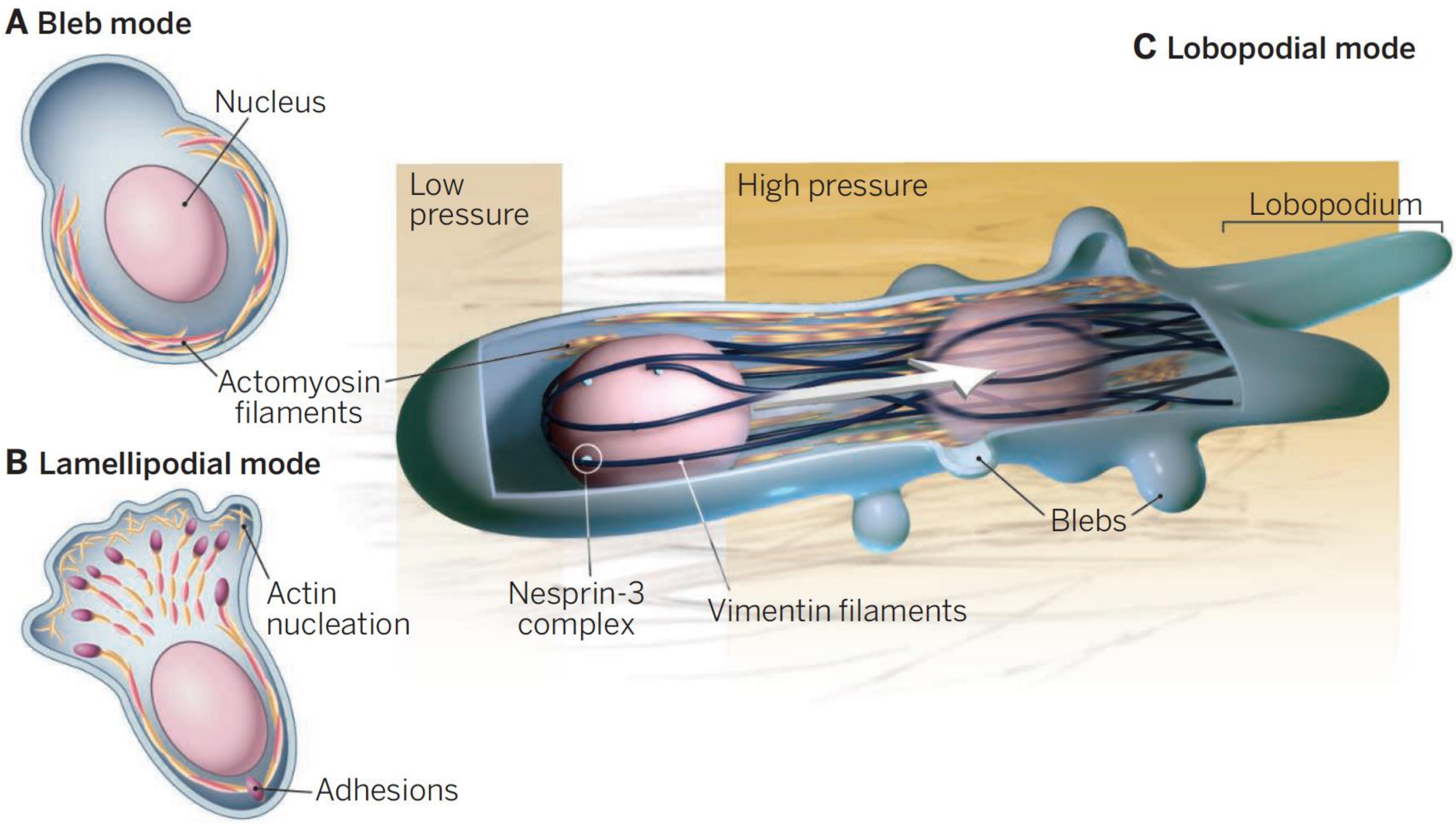
Retrograde flow







Lobopodial mode of migration



Nuclear piston

- Actomyosin contractility pulls the nucleus forward and pressurize the front of the cell

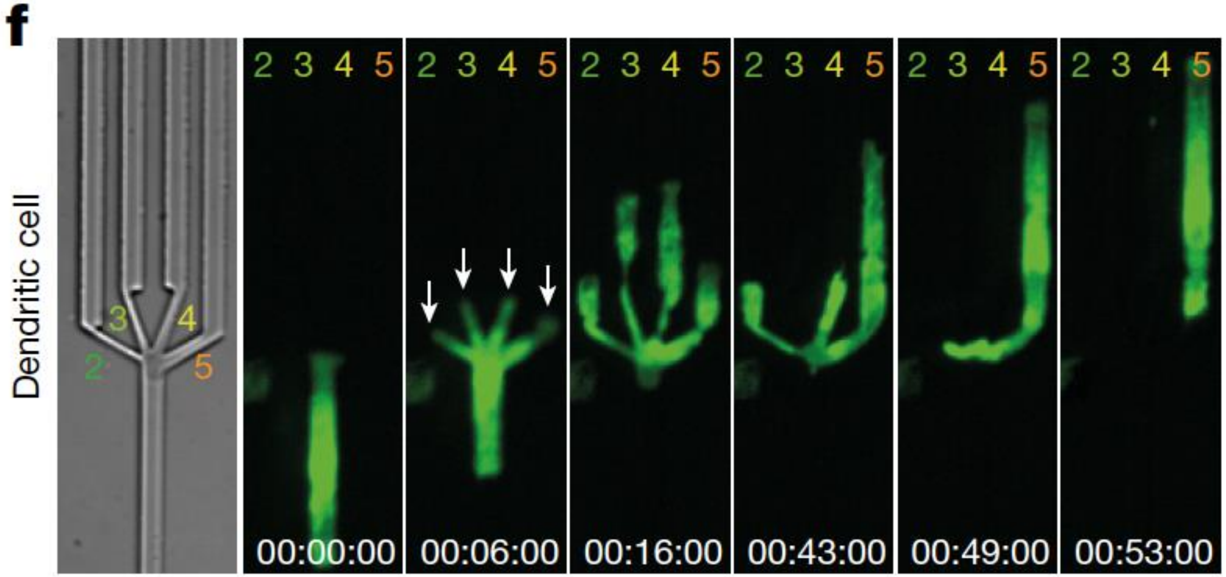
- Hydrostatic pressure
  - Cell posterior: 900 Pa
  - Lobopodia: 2400 Pa
  - Lammellipodia migration: 500 Pa

- **Servo-null method**: machine detects pressure-induced changes to the resistance of a microelectrode inserted into the cytoplasm. The system adjusts the air pressure behind the electrolyte solution in the microelectrode to compensate for any external pressure-induced resistance change. Compensation pressure is the intracellular pressure.

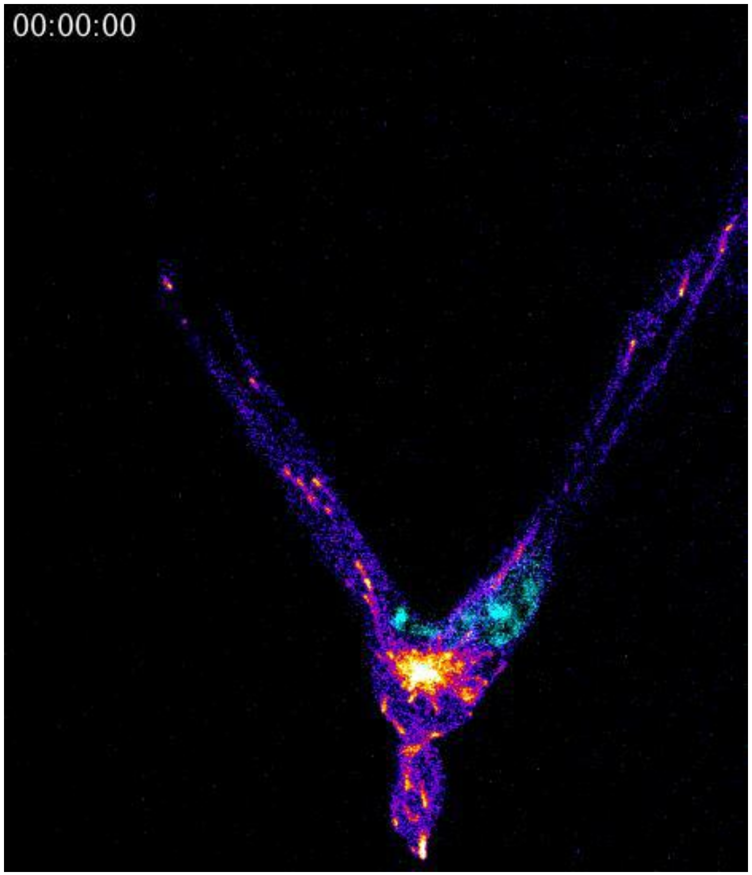
Nucleus as a mechanical gauge

- Amoeboid cells: nucleus first configuration
- Mesenchymal cells: cell body first configuration

- Amoeboid cells use the nucleus to rapidly navigate along the path of least resistance
- Once the nucleus and the microtubule organizing center pass the largest pore, cytoplasmic protrusions in smaller pores are retracted



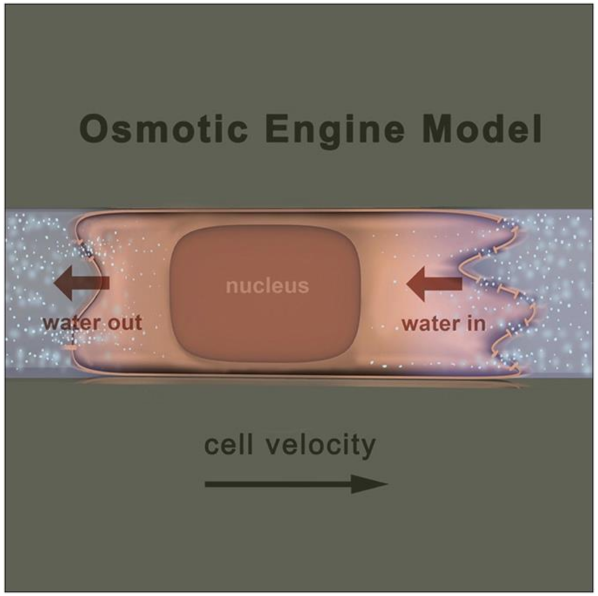
Nucleus as a mechanical gauge

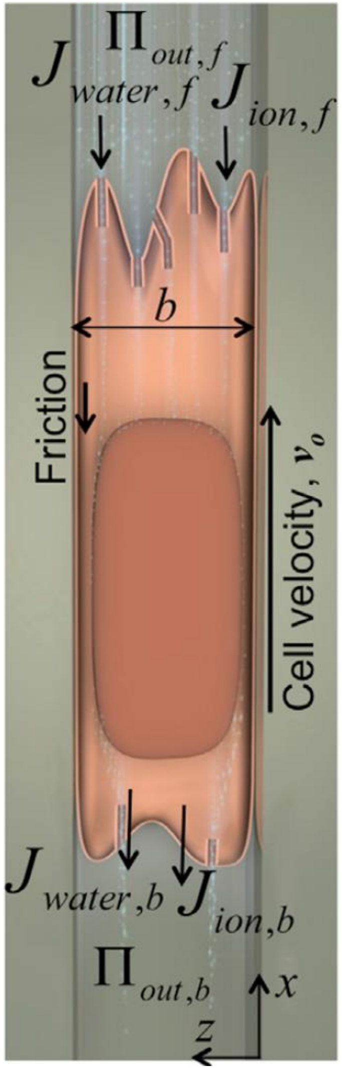


Nuclear Dynamics

in
3D Collagen Gels

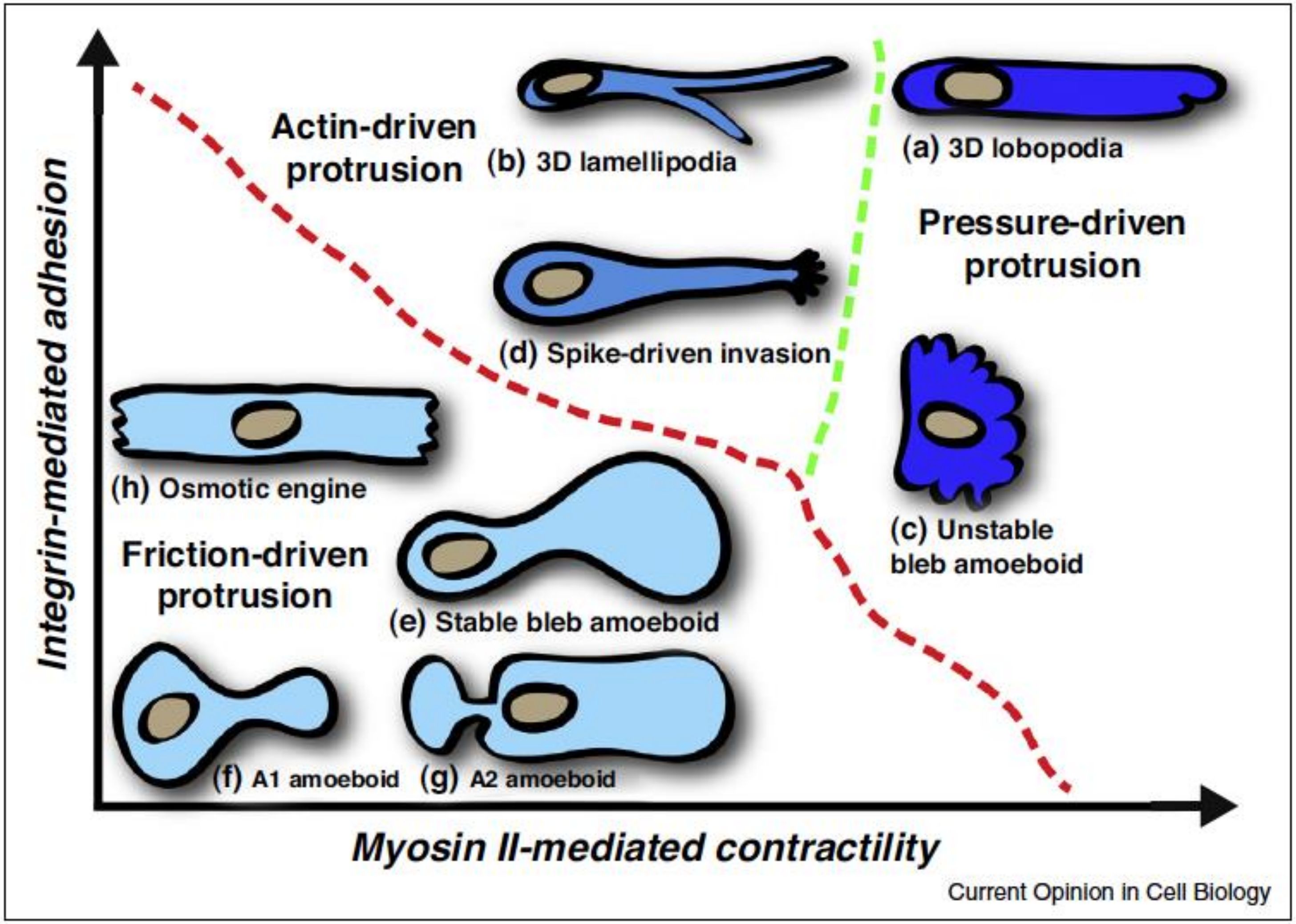
Osmotic engine





- The water flow across a semipermeable membrane
  - Chemical potential across the surface
  - Equilibrium: Hydrostatic pressure difference balances the osmotic pressure difference
  - Cells can direct water flow at the leading and trailing edge

Adaptive features and plasticity



Adhesive vs non-adhesive migration