Week 05: Adhesion and mechanotransduction

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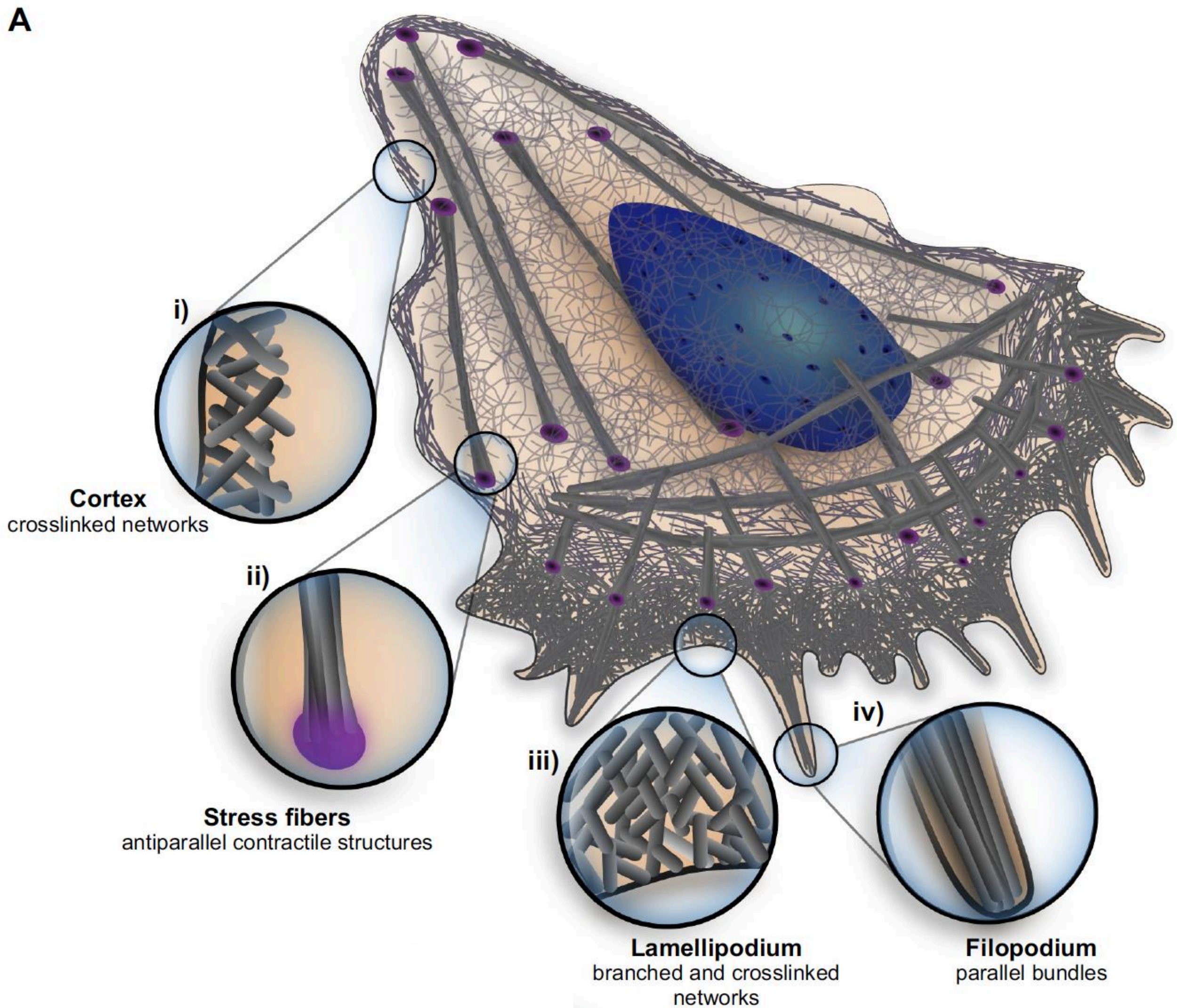
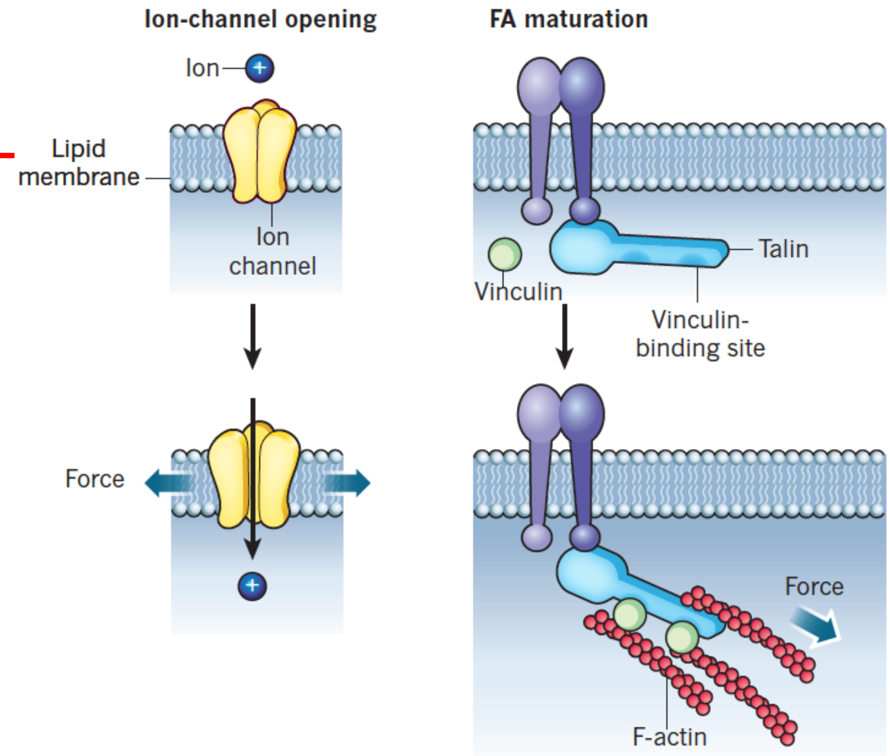
Actin Dynamics, Architecture, and Mechanics



Mechanotransduction



- Stretch-activated ion channels
- Adhesion complexes
- Cell-cell junctions
- Cytoskeletal components
- Nuclear mechanotransduction

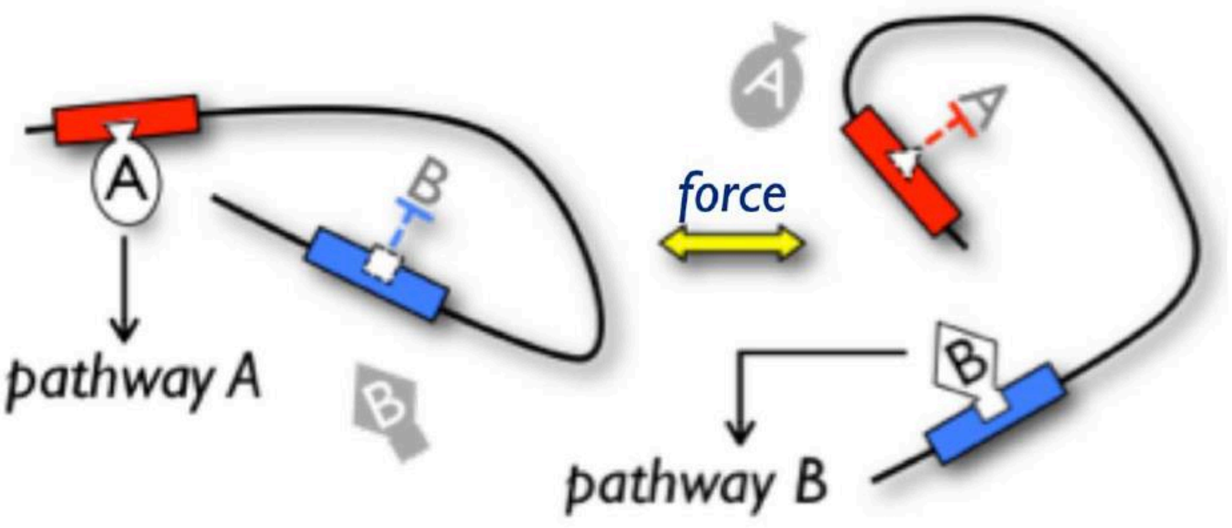
- Cellular signaling pathways
  - Rho-family GTPases
  - Mitogen-activated protein kinase and extracellular signal-regulated kinase (MAPK-ERK)
  - Nuclear translocation of transcriptional regulators (YAP/TAZ)

- Expression of mechanosensitive genes

Mechanotransduction

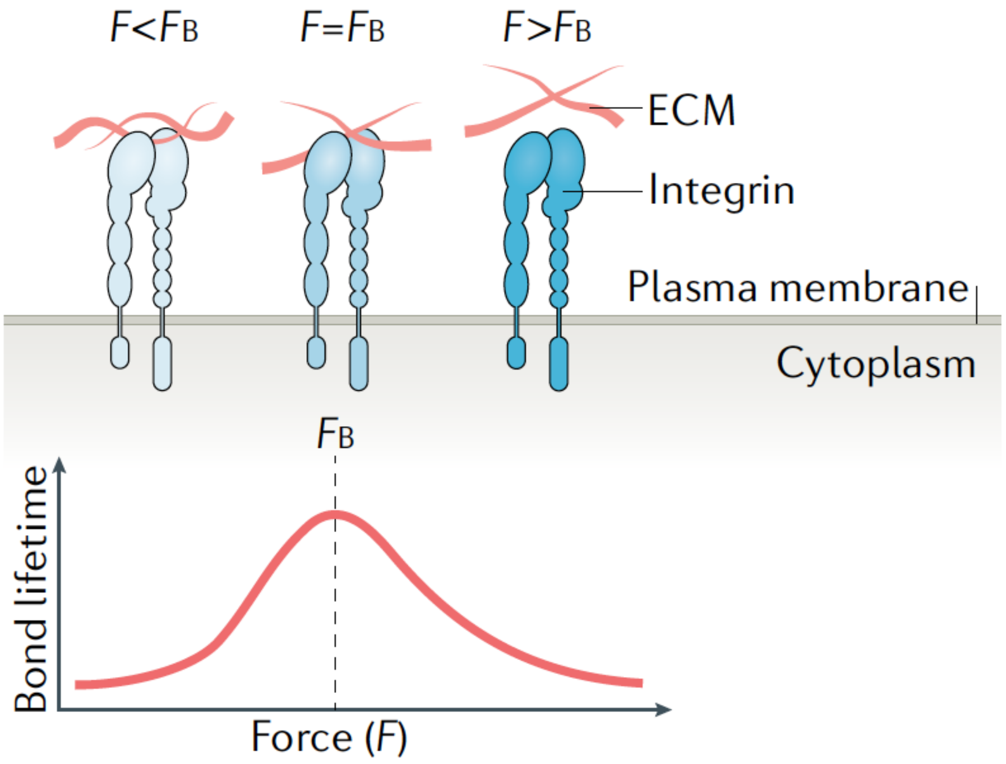
- Specific proteins undergo force-induced alterations in conformation
  - Changes in catalytic activity
  - Affinity to binding partners (catch and slip bond)

- Mechanical force can shift the equilibrium between pre-existing states
  - One myosin molecule: 2pN



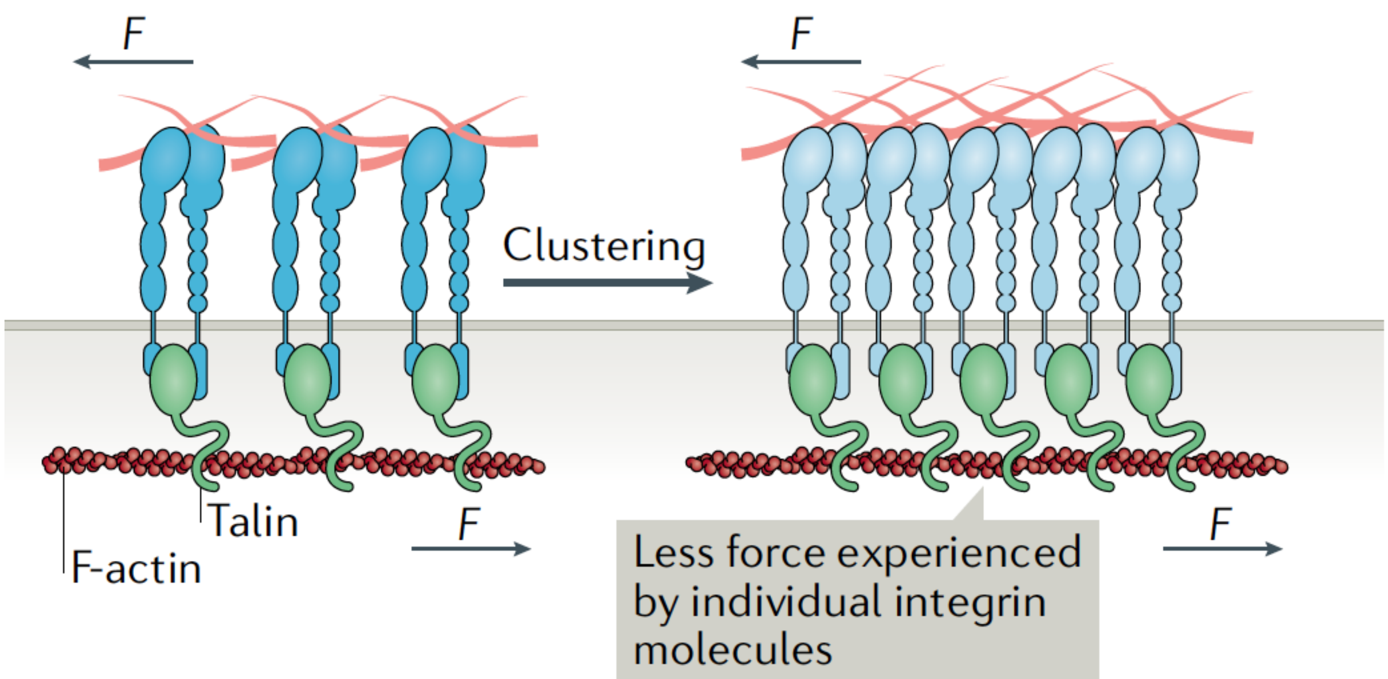
Catch bond

- Unbinding rates decrease with applied force up to a given threshold
- Above that thresholds, unbinding rates increase (slip bond)
- Optimal stability: minimum unbinding rate

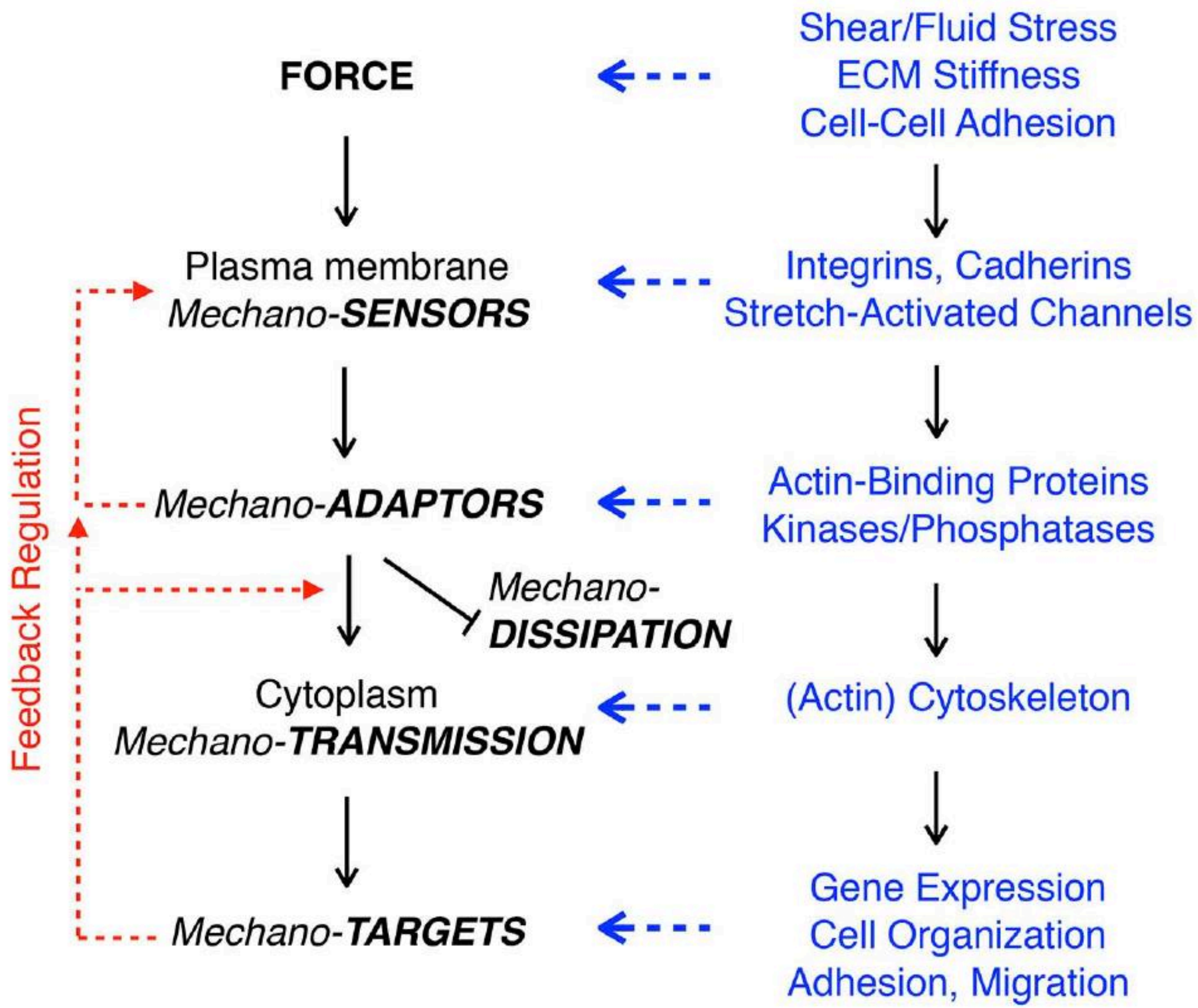


Receptor clustering

- Clustering into adhesion complexes
- Maybe facilitated by controlled diffusion
  - Ligand-bound integrins are constrained
- Minimizes elastic energy by decreasing the applied strain

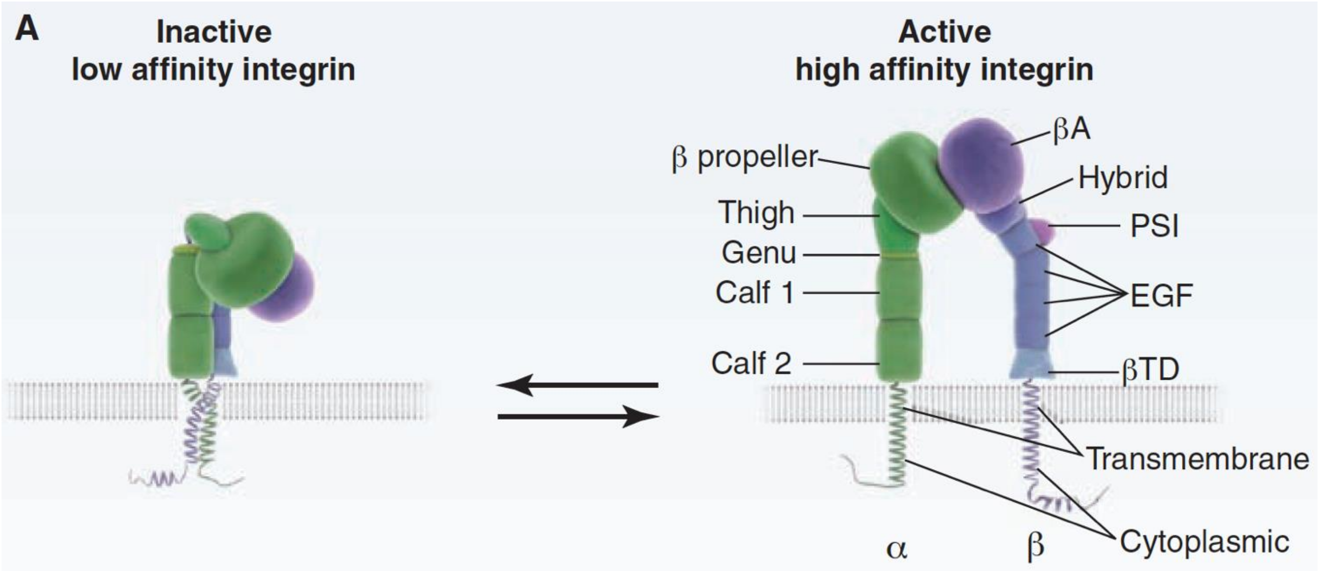


Functional organization of the genome



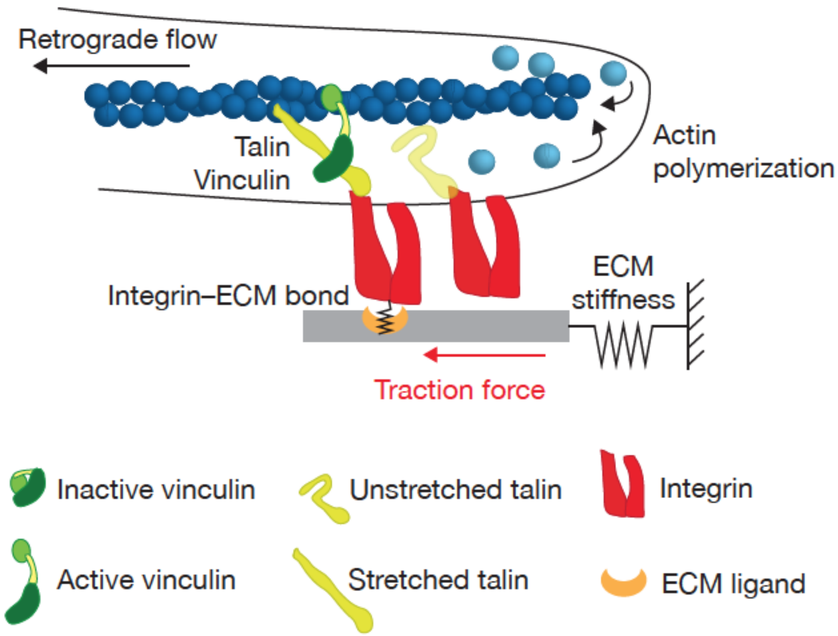
Integrins

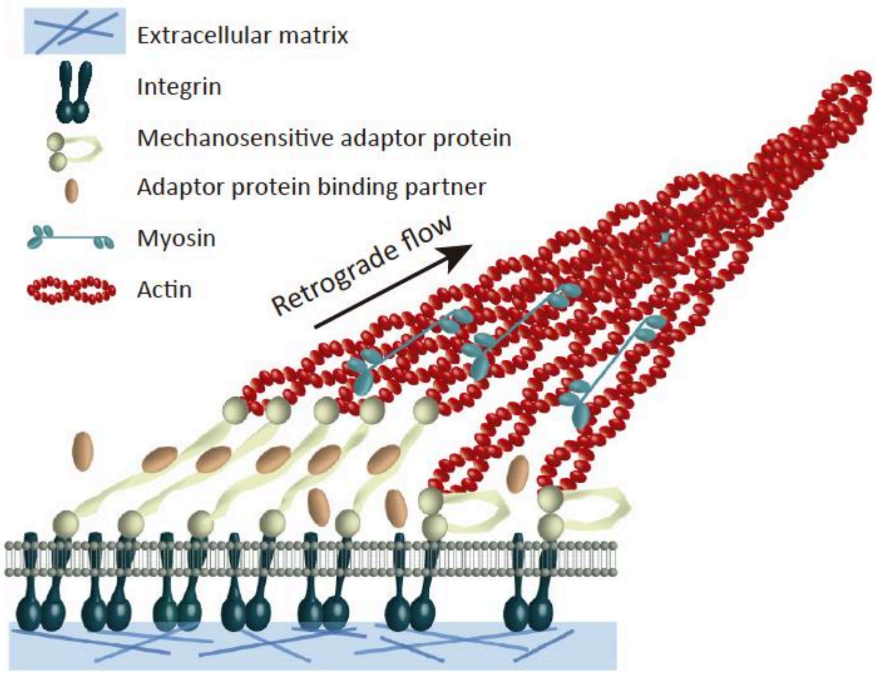
- Transmembrane receptors
- Found in all animals
- Several types exist, one cell generally has multiple different types
- Upon ligand binding, integrins activate signal transduction pathways
- Ligands include
  - Fibronectin (>8 integrins), collagen, and laminin (>5 integrins)
- The name was inspired by its function: integrator



Integrin-mediated mechanotransduction

- Integrins connect the extracellular matrix with F-actin cytoskeleton
- Transduce forces generated by the actin retrograde flow and myosin II to the ECM through focal adhesion proteins
- Force alters the functions of mechanosensitive proteins
- Rapid responses in cellular mechanics
- Long-term changes in gene expression





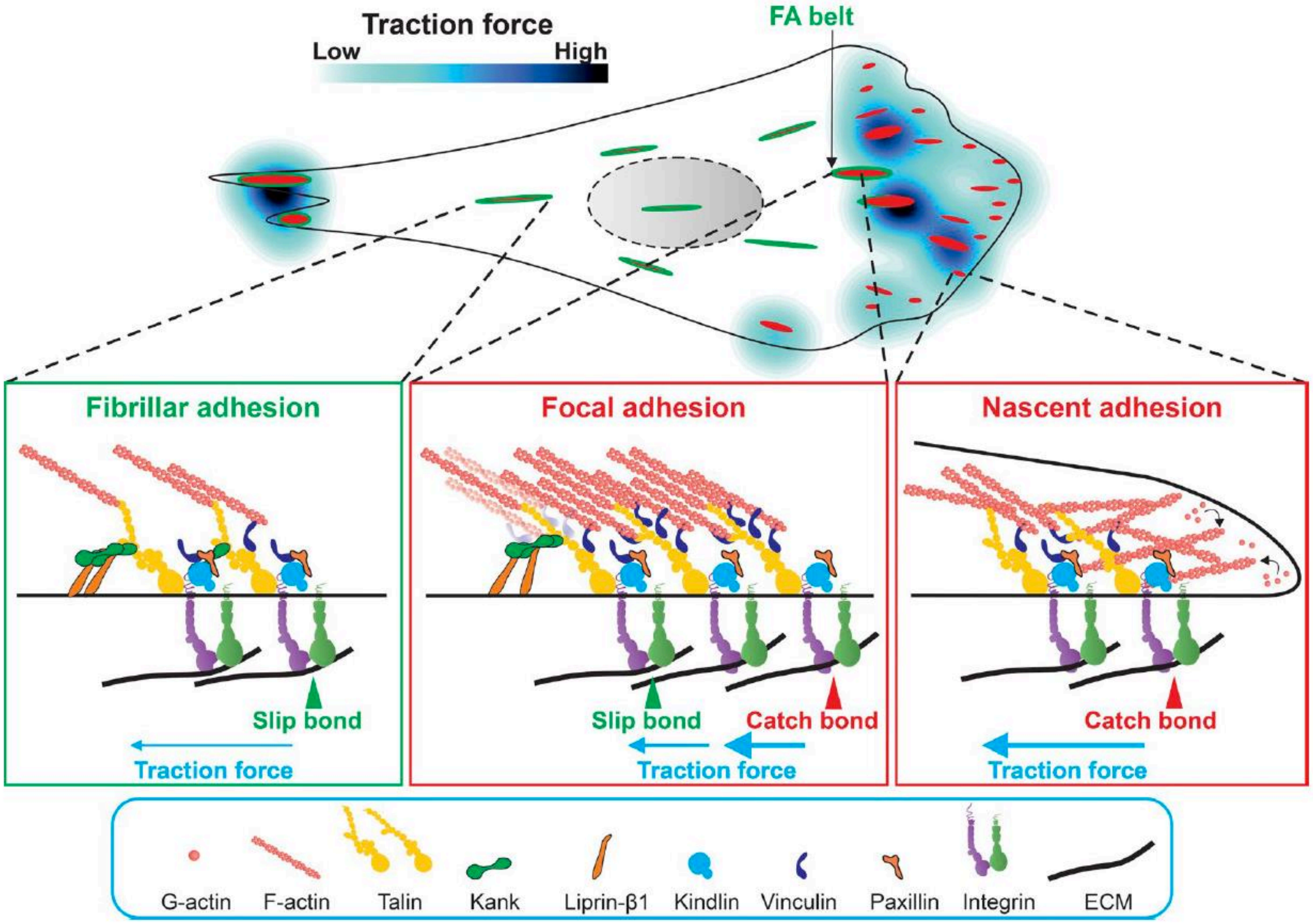
Molecular clutch hypothesis

- Mechanical linkage formed by dynamic associations between
  - ECM-bound integrins
  - Force-generating actomyosin cytoskeleton

- The clutch is mediated by talin and vinculin
- Talin directly interacts with the cytoplasmic domain of activated integrins and F-actin
- Vinculin binds to talin and F-actin to strengthen connections

- Clutch is disengaged
  - Retrograde actin flow is fast and traction forces are very low
- Engagement of the clutch
  - The kinetic power of the actin retrograde flow and actomyosin contractility are converted into traction forces
  - Polymerizing F-actin pushes the resistant plasma membrane at the leading edge forward → protrusions

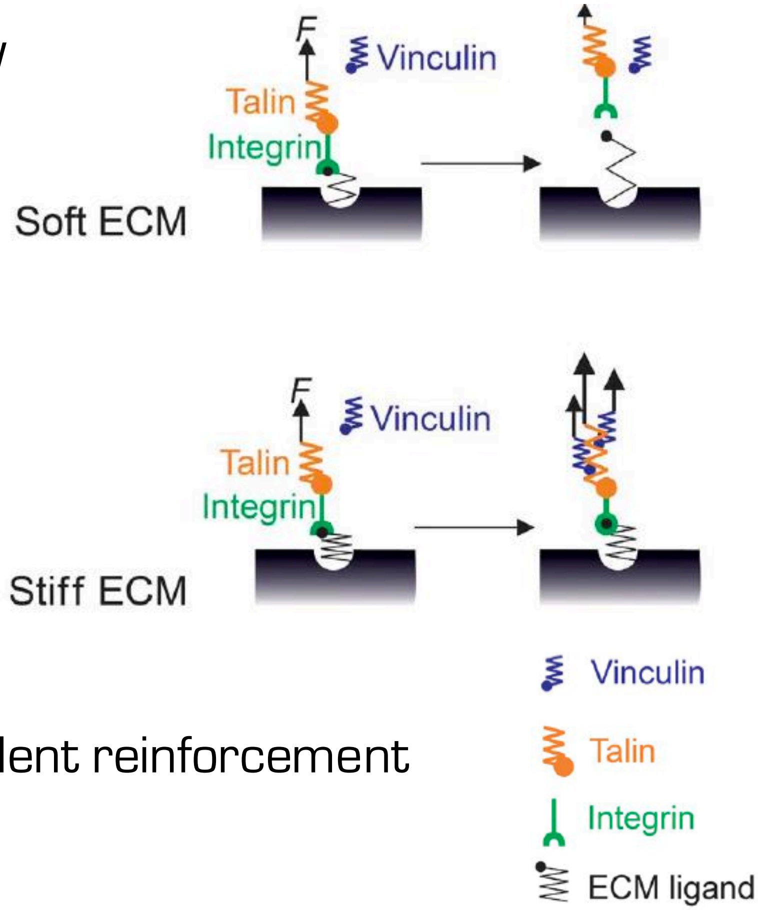
Molecular clutch hypothesis



Molecular clutch hypothesis

- Molecular clutch is sensitive to ECM rigidity

- **On stiff substrates**
    - Fast mechanical loading rates
    - Protein unfolding of talin
    - Expose cryptic sites
    - Vinculin binding and reinforcement
    - Higher forces
- **On soft substrates**
    - Substrate easily deforms
    - Slow mechanical loading rate
    - Not sufficient to induce vinculin-dependent reinforcement
    - Transmission at low level



- Frictional Slippage and Stick-Slip rather than static connections

Molecular clutch hypothesis

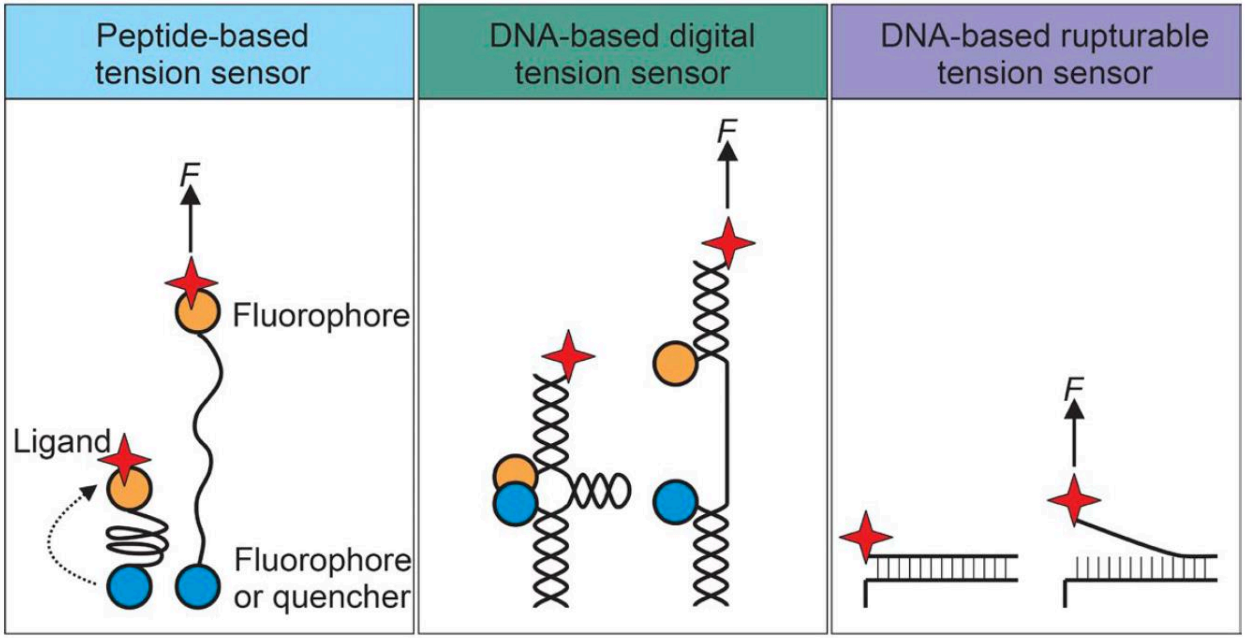
- Myosin motors contract actin filaments at a fixed speed (120 nm/s) if their action is unopposed by force
- If a force opposes myosin action, contraction speed will decrease with force until stalling completely at 2 pN
- Force transmitted to molecular bonds increase the lifetime
- Binding and unbinding rates

- Bonds fail at high force: catastrophic event or stick-slip
- Substrate controls the loading rate
- Unbinding rates become faster than binding rates above optimal rigidity
    - Number of clutches simultaneously engages drops drastically
    - Frictional slippage

- Biphasic relationship between rigidity (loading rate) and force
- First increase and then decrease

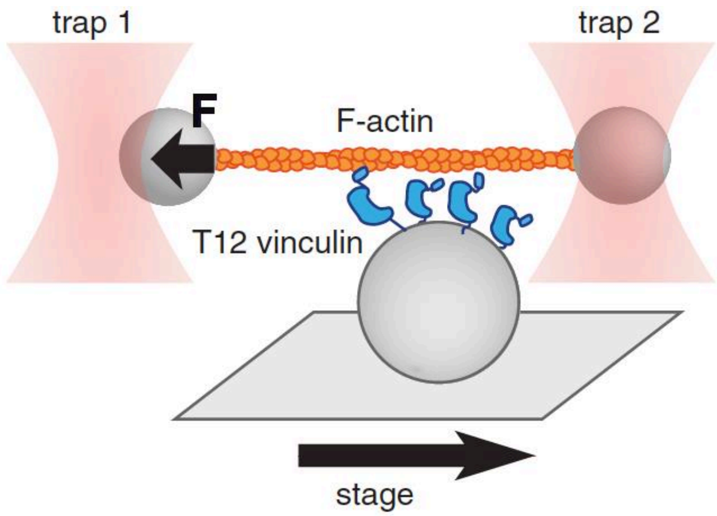
Molecular tension sensors

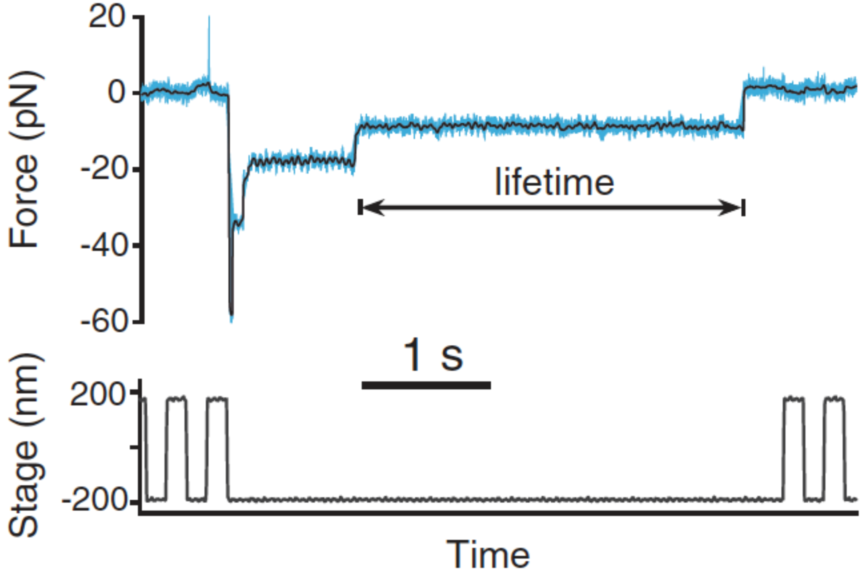
- Piconewton accuracy
- Change in fluorescence signal
- Integrins apply forces
  - between 1 and 5 pN
  - DNA-based sensors: up to 15 pN
  - Rupture: up to 40 pN



Vinculin forms a catch bond with F-actin

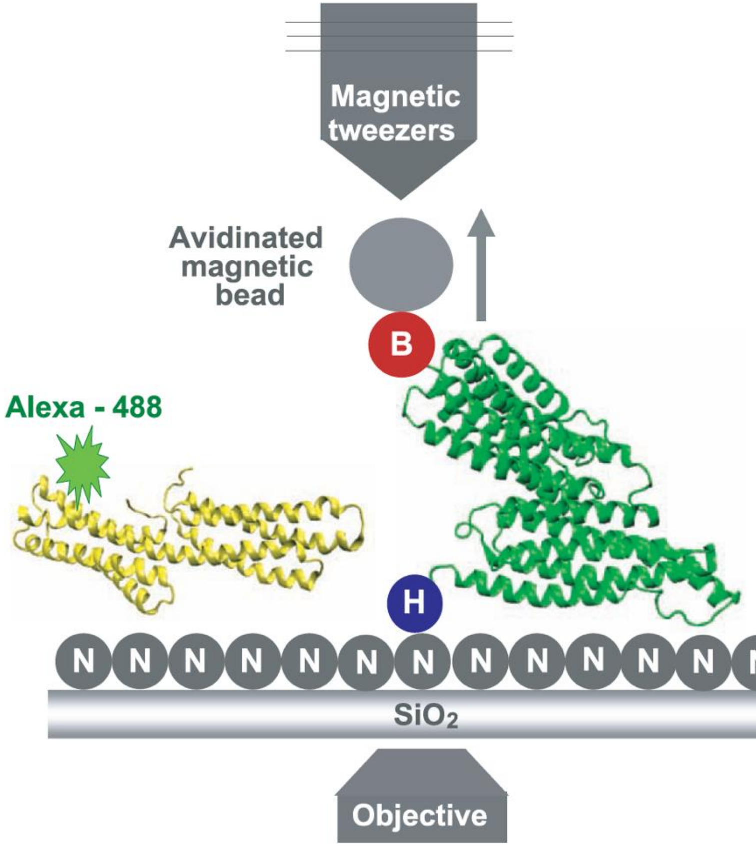
- Force toward pointed end of the actin filament resulted in a bond that was maximally stable at 8 pN with a mean lifetime of 12 seconds
- Directional and force-stabilizing binding of vinculin to F-actin





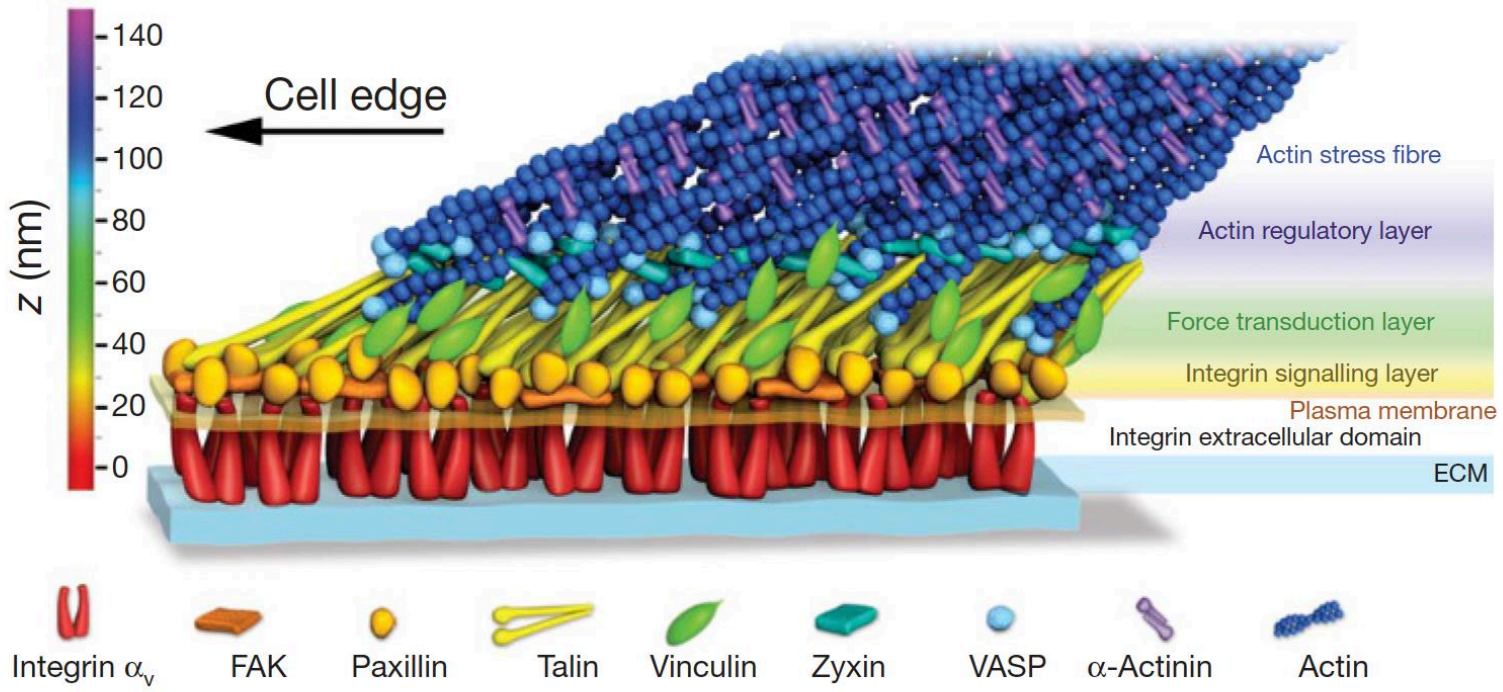
Stretching single talin rod molecules

- Force extension experiment with MT
  - Unfolding and binding events
- Constant calibrated force with AFM
  - Unfolding rate
  - Up to 150 pN



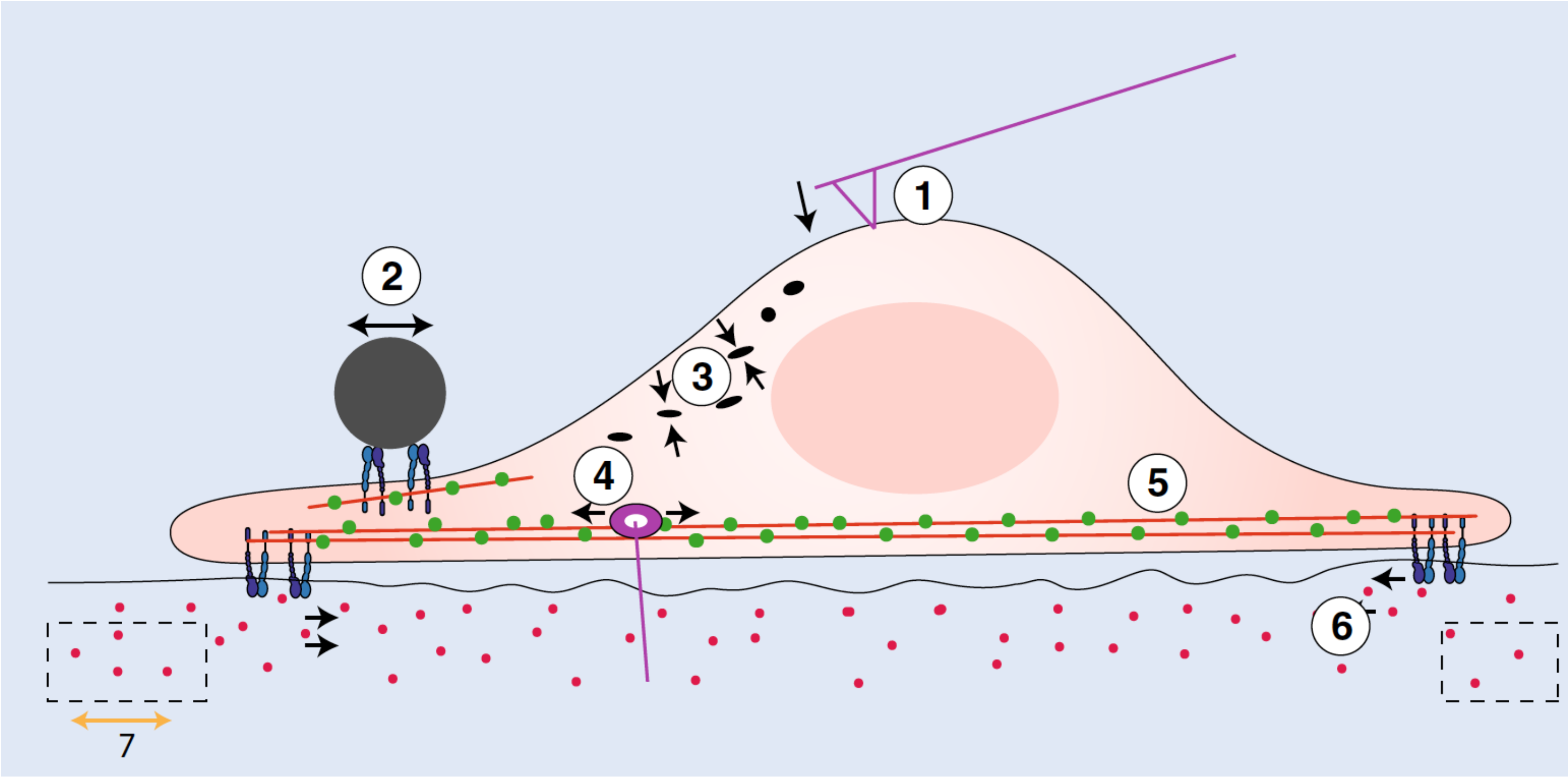
3D nanoscale architecture of focal adhesions

- Super resolution fluorescence microscopy
  - Photoactivated localization microscopy (PALM)



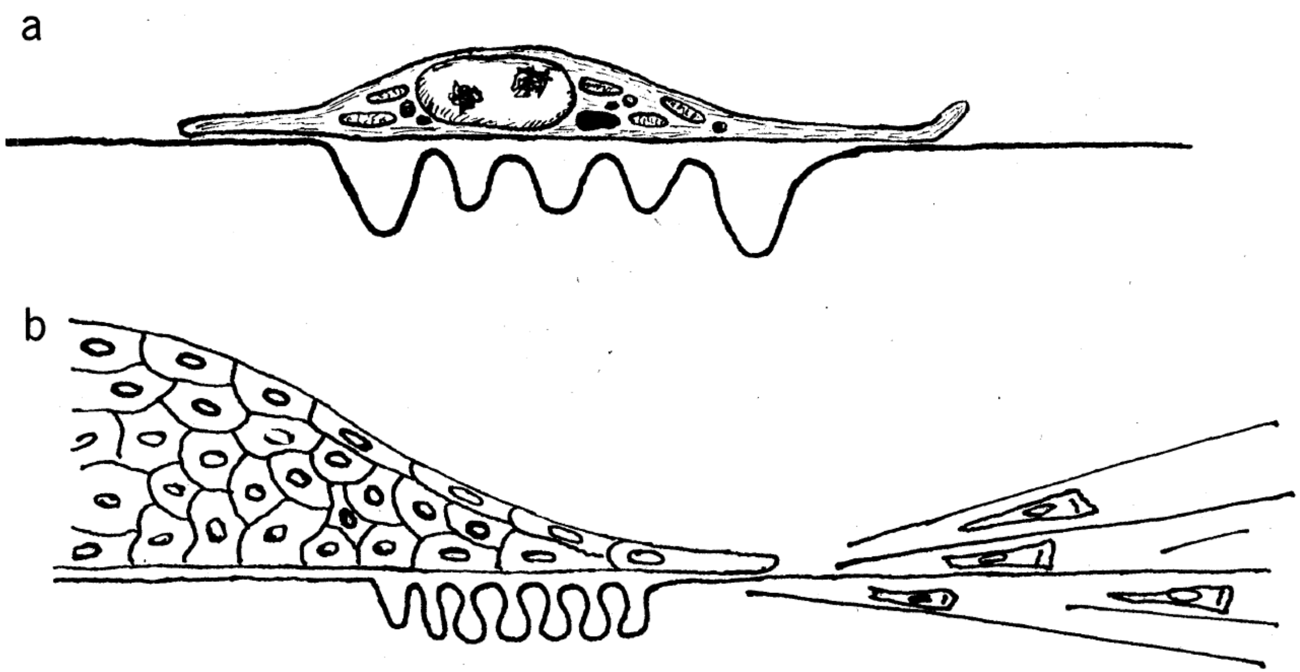
Methods for measuring cellular forces

- AFM, tweezers, droplets/gels, laser cutting, time-lapse imaging, traction force microscopy, actuated substrates, wound healing assays



Traction force microscopy: Silicone rubber substrate

- When cells are cultured on very thin sheets of cross-linked silicone fluid, the traction forces are made visible as elastic distortion and wrinkling of the substrate (Science, 1980)



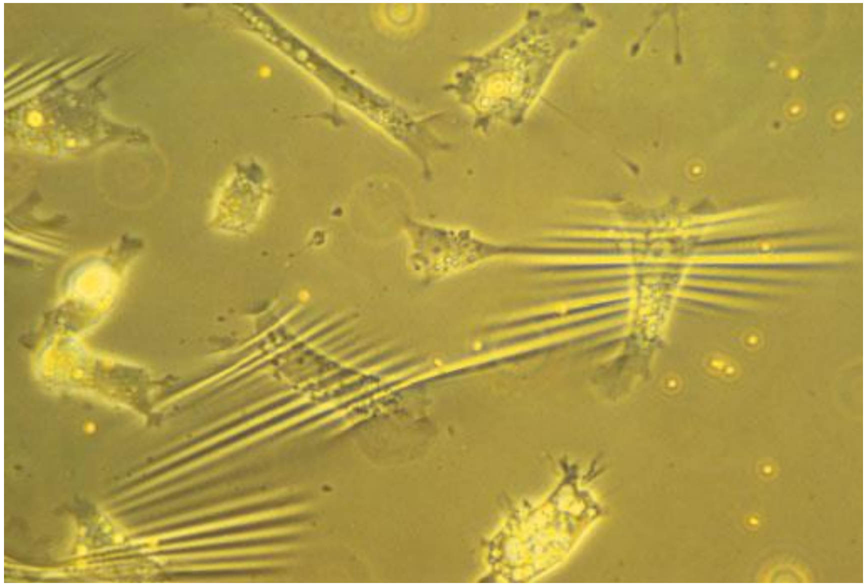
Traction force microscopy: Silicone rubber substrate

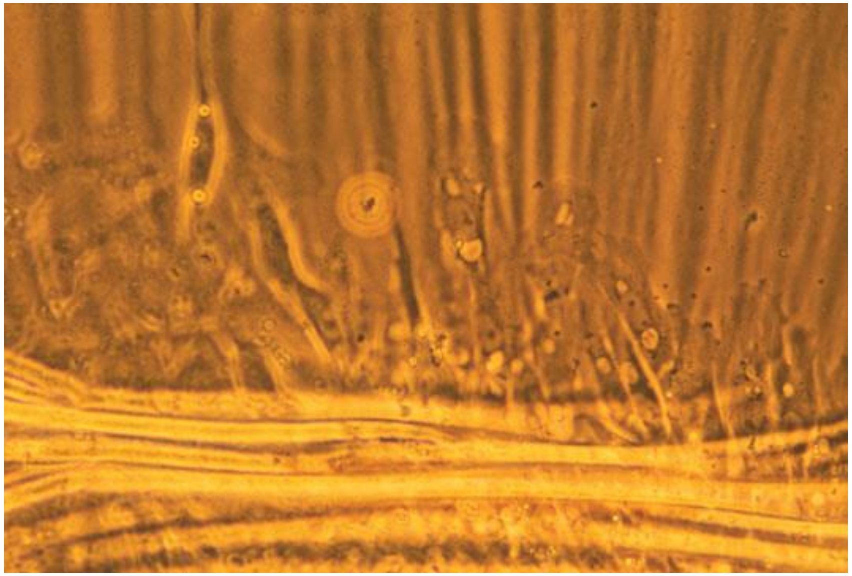
- Specifications of the material
  - As inert as possible
  - Nontoxic
  - Transparent
  - Shows sufficiently little strain-induced birefringence
  - Refractive index does not depend on the polarity of the light
  - Dogma on dehydration (goes back to Paul Weiss)

- **Solution**: brief exposure of silicone fluid to a flame
  - Crosslinks the outermost layer
  - 1 μm thick skin on an un-crosslinked fluid that serves as a lubricant

Traction force microscopy: Silicone rubber substrate

- Cells slowly pull the rubber sheet centripetally past their lower surface
- Produce pronounced winkles
- The sheet quickly expands to its original shape when the cells are detached
- Measure shear forces using a calibrated glass microneedle
- Shear forces on the order of 0.001 dyne/μm (1 dyne = 10 μN)





Traction force microscopy: Silicone rubber substrate

Traction force microscopy: Silicone rubber substrate

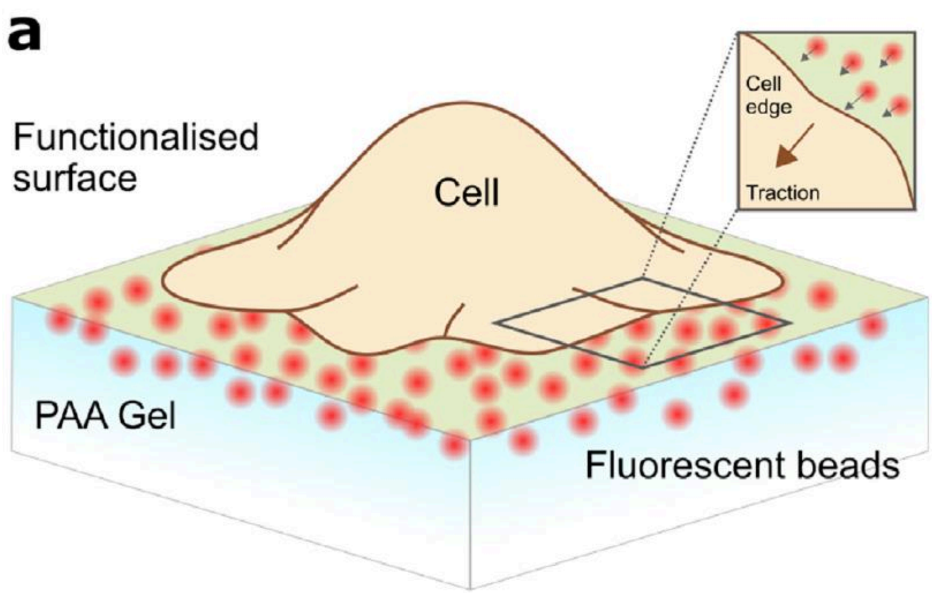
- Wrinkles in silicone substrate are usually larger than the cells generating them
- Winkles develop very slowly
- Wrinkles are intrinsically nonlinear and chaotic

- Very limited spatial and temporal resolution

Traction force microscopy: polyacrylamide substrate

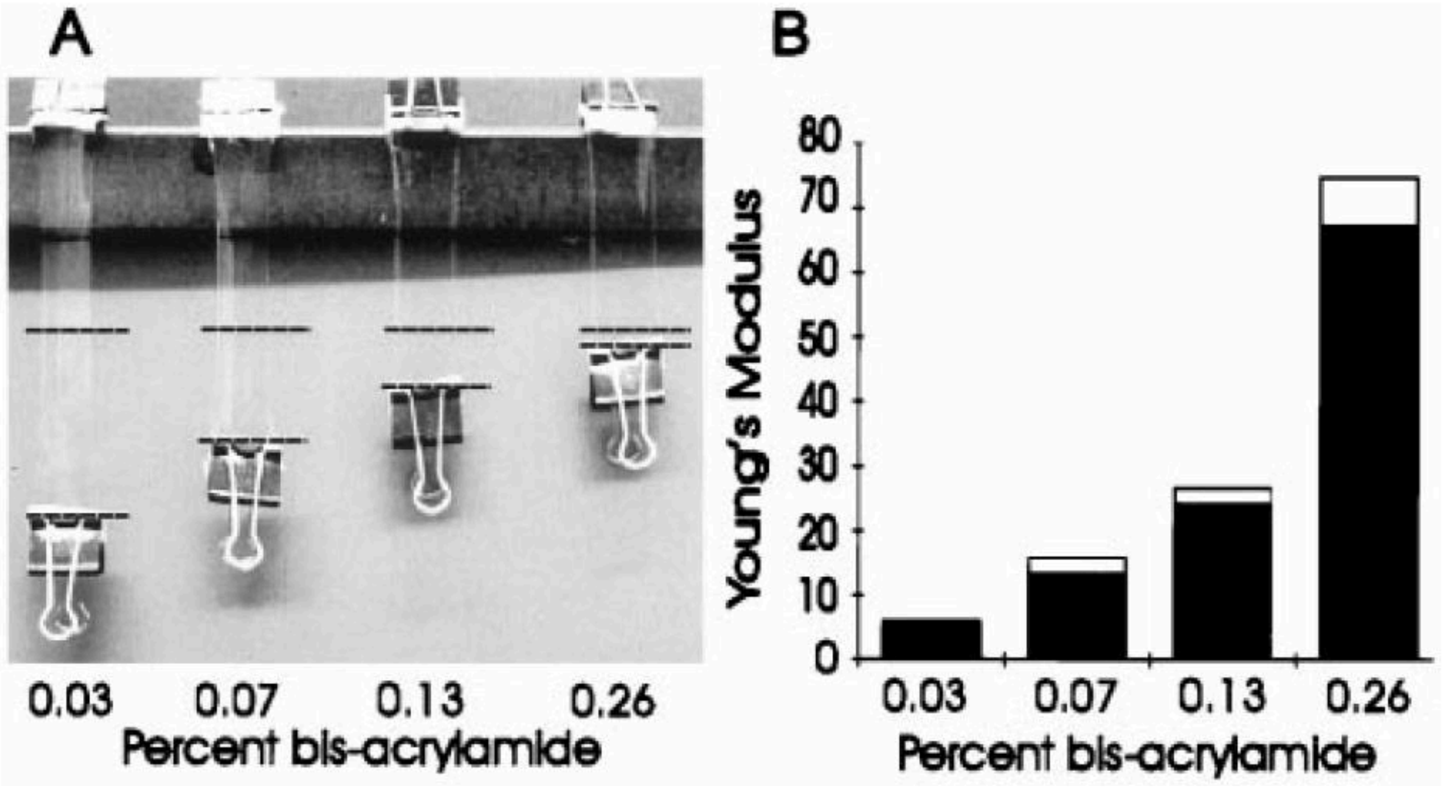
- Linearly elastic material
- Tunable stiffness
  - Variations of monomer and cross-linker concentrations

- 70 μm thick layer covalently bonded to glass coverslip
- Fluorescent latex makers beads randomly distributed throughout the gel
- The surface of the gel is covalently decorated with type I collagen



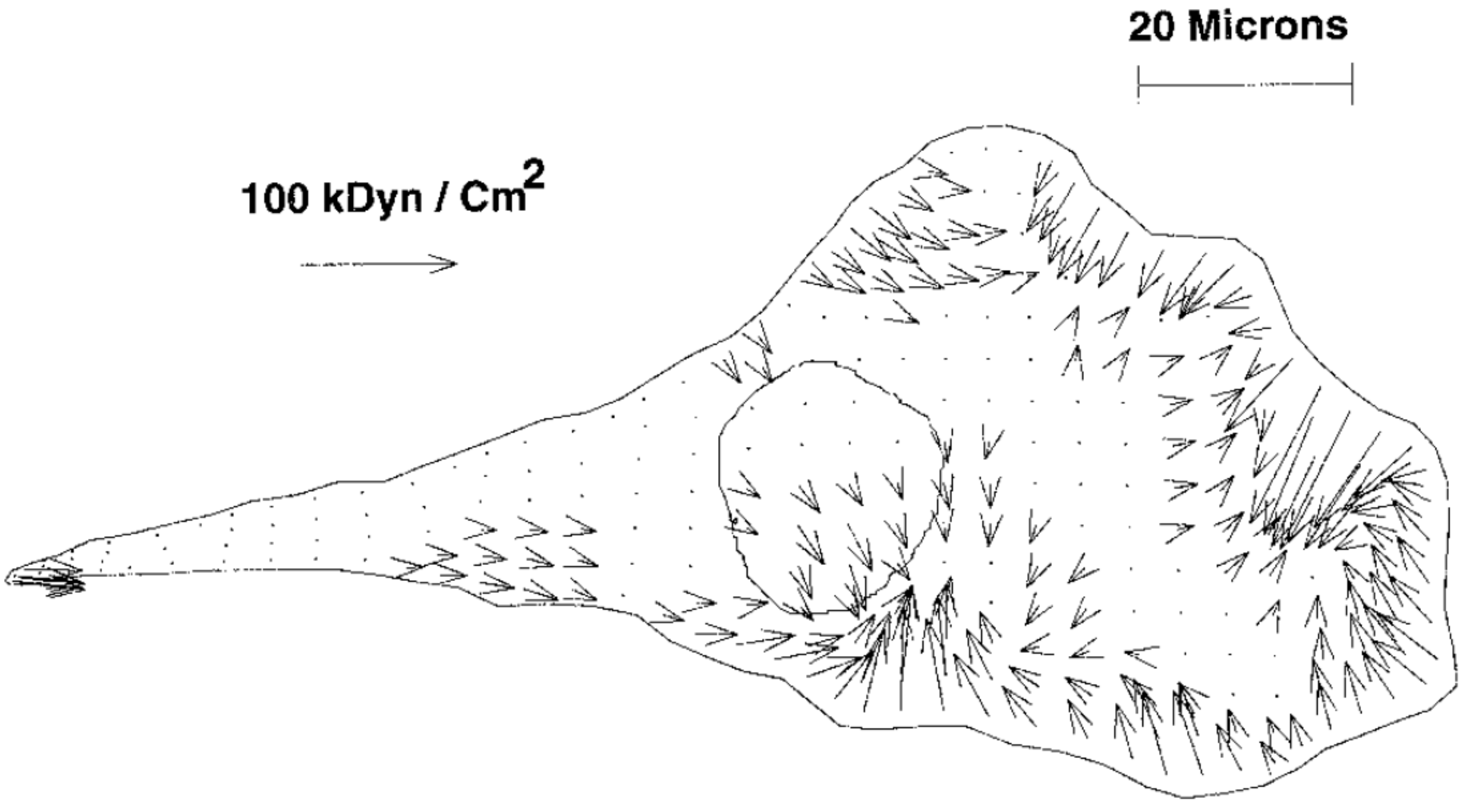
Traction force microscopy: polyacrylamide substrate

- The unit of Young's modulus is kPa



Traction force microscopy: polyacrylamide substrate

- 200 RMS bead displacements (Biophysical Journal, 1999)
- Propulsive thrust: 1 μN

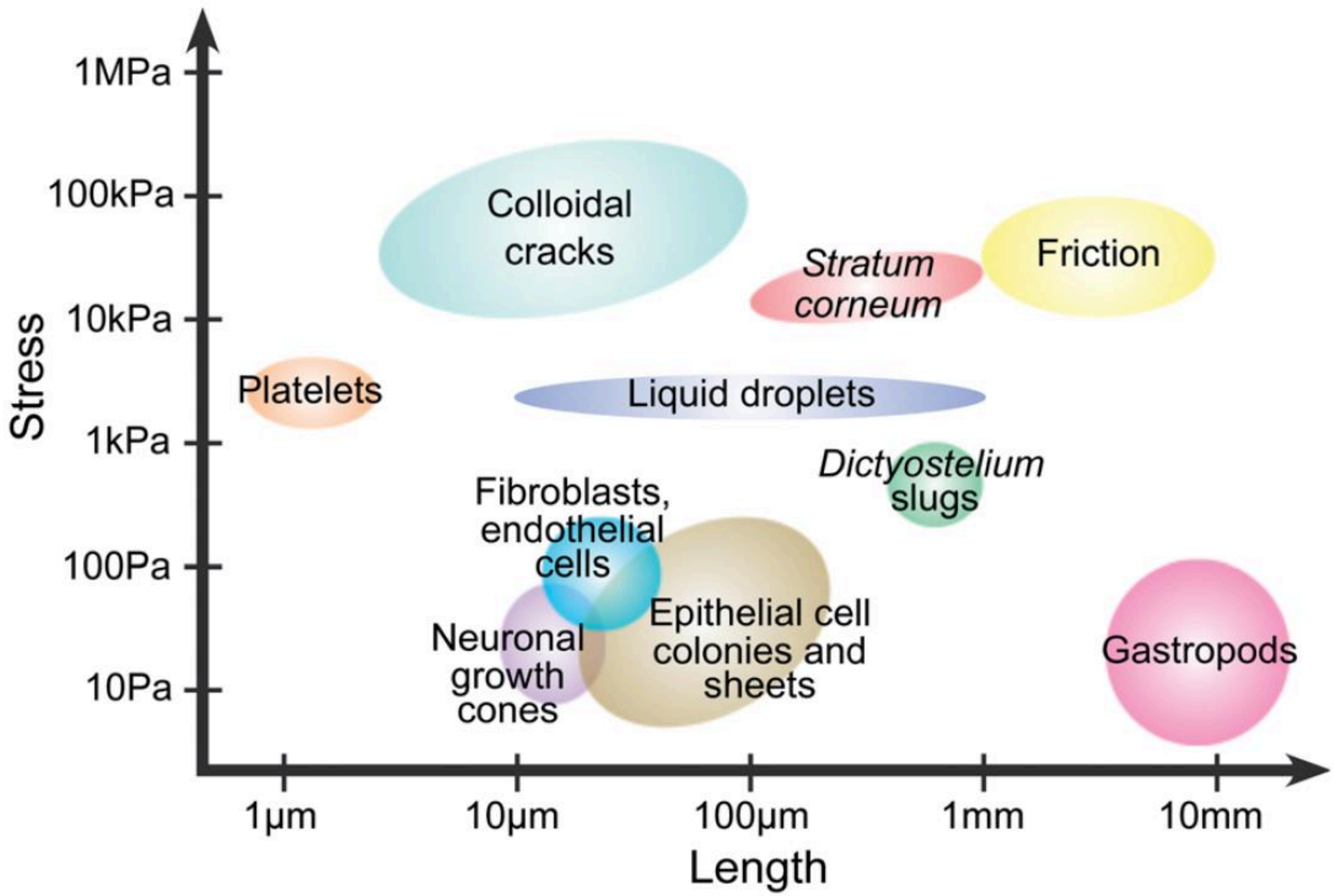


Traction force microscopy: polyacrylamide substrate

- An attractive hypothesis is that, when receptors become anchored to a rigid substrate or cross-linked, the resistance to cytoskeleton-generated forces causes an increase in tension at adhesion structures and activates downstream signals through a force-sensitive enzyme complex.
- However, to detect flexibility, it is necessary for the cells to modulate and measure the probing force in response to different substrate resistance (otherwise, cells will simply deform soft substrates to an increasing extent until they experience a similar resistance as on stiff substrates).

- Alternatively, cells may be able to measure the amount of substrate deformation as they apply a defined probing force.

Traction force microscopy: polyacrylamide substrate



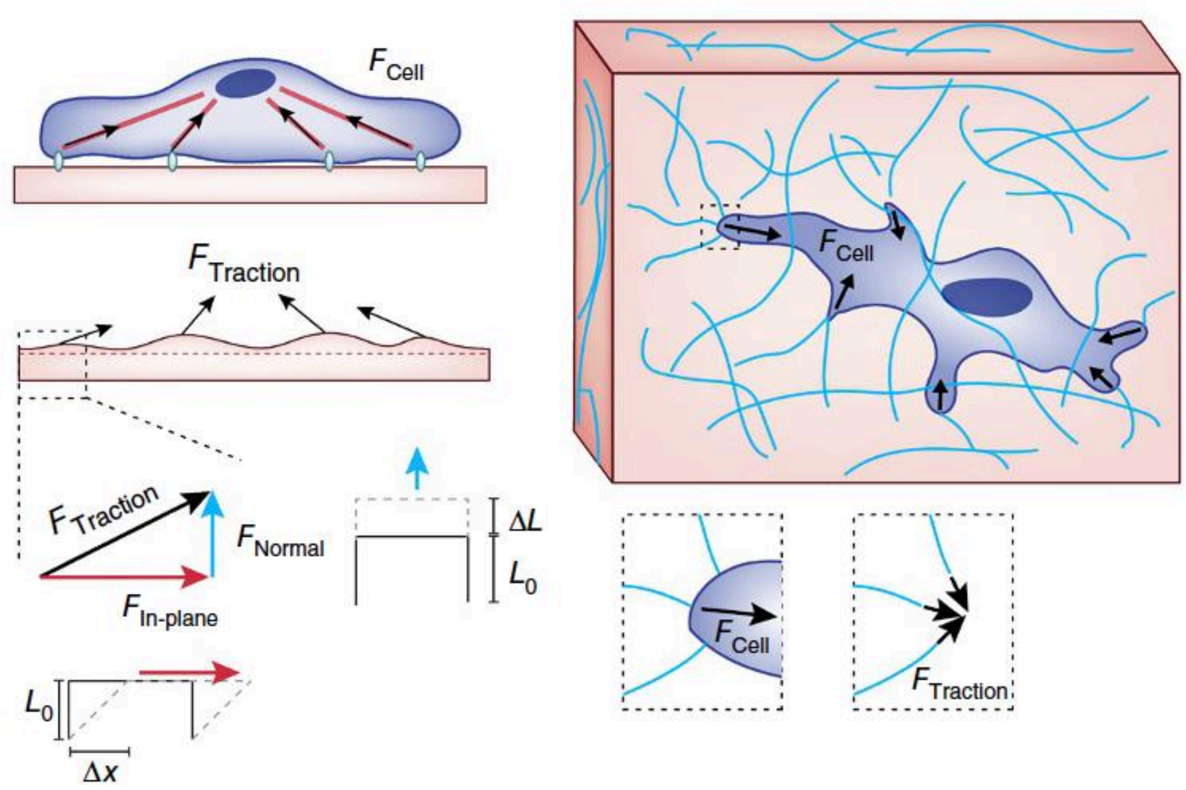
Super-resolved traction force microscopy

- Twofold increase in spatial resolution (up to 500 nm)
- Takes a few minutes to gather sufficient information on markers

Technique	Resolution (spatial/force)	Geometry	Magnitude/direction of force
FRET force sensors	Single molecule/10s pN	2D	Magnitude only
Micropillar	1–2 μm/50 pN	2D	Magnitude and direction
TFM	1–2 μm/1 nN	2D and 3D	Magnitude and direction
STFM	0.4–1 μm/<1 nN	2D	Magnitude and direction

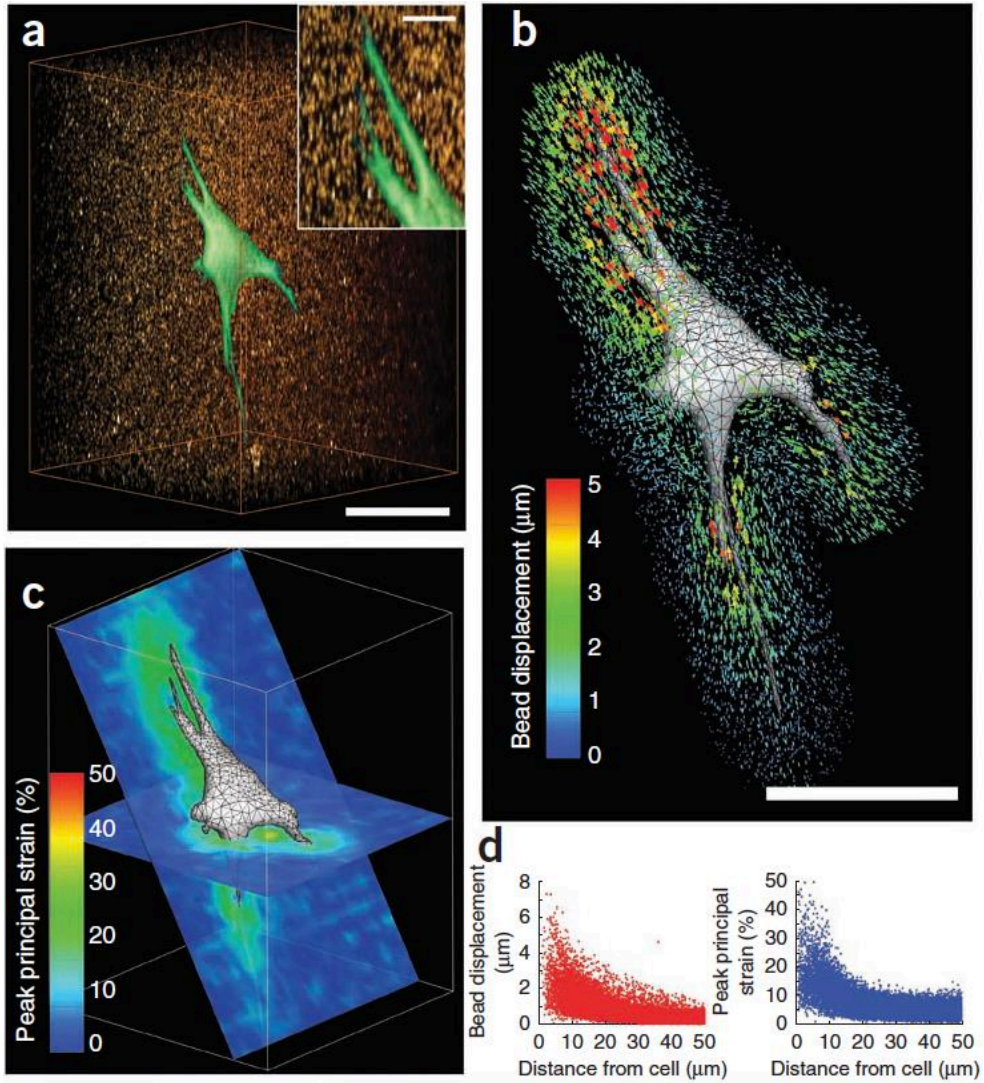
From 2D to 3D

- 2.5D TFM to quantify the normal forces
- 3D scaffolds to monitor forces in all dimensions

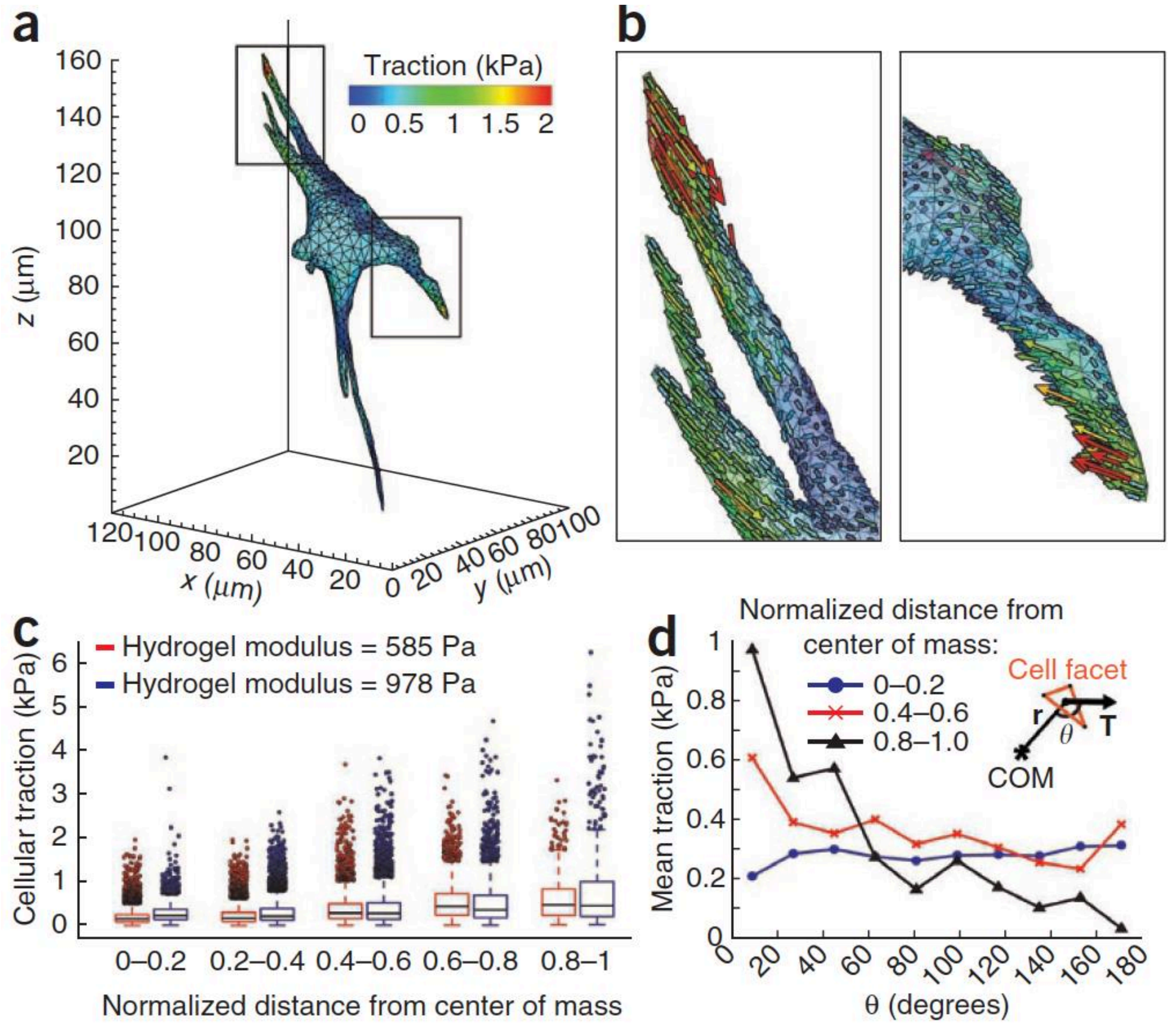


Traction force microscopy: PEG hydrogel matrix

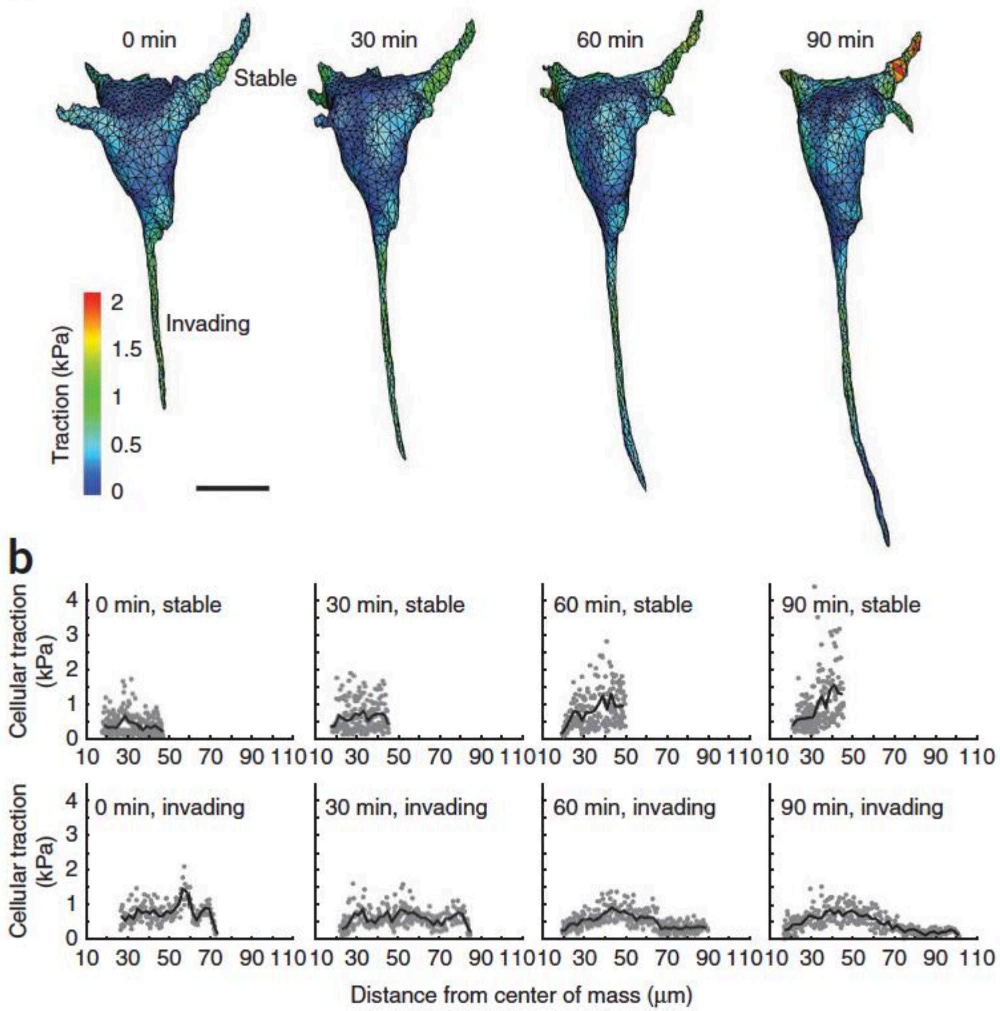
- Proteolytically degradable domains in the polymer
- Pendant adhesive ligands
- Cell can invade and spread
- Young's modulus 0.6-1kPa
- Tracking displacements of 80,000 fluorescent beads
- Up to 50% strain in the vicinity of slender extensions
- Linear elasticity and FEM
- 4h to calculate from a single dataset



Traction force microscopy: PEG hydrogel matrix



Traction force microscopy: PEG hydrogel matrix



Traction force microscopy: collagen gel

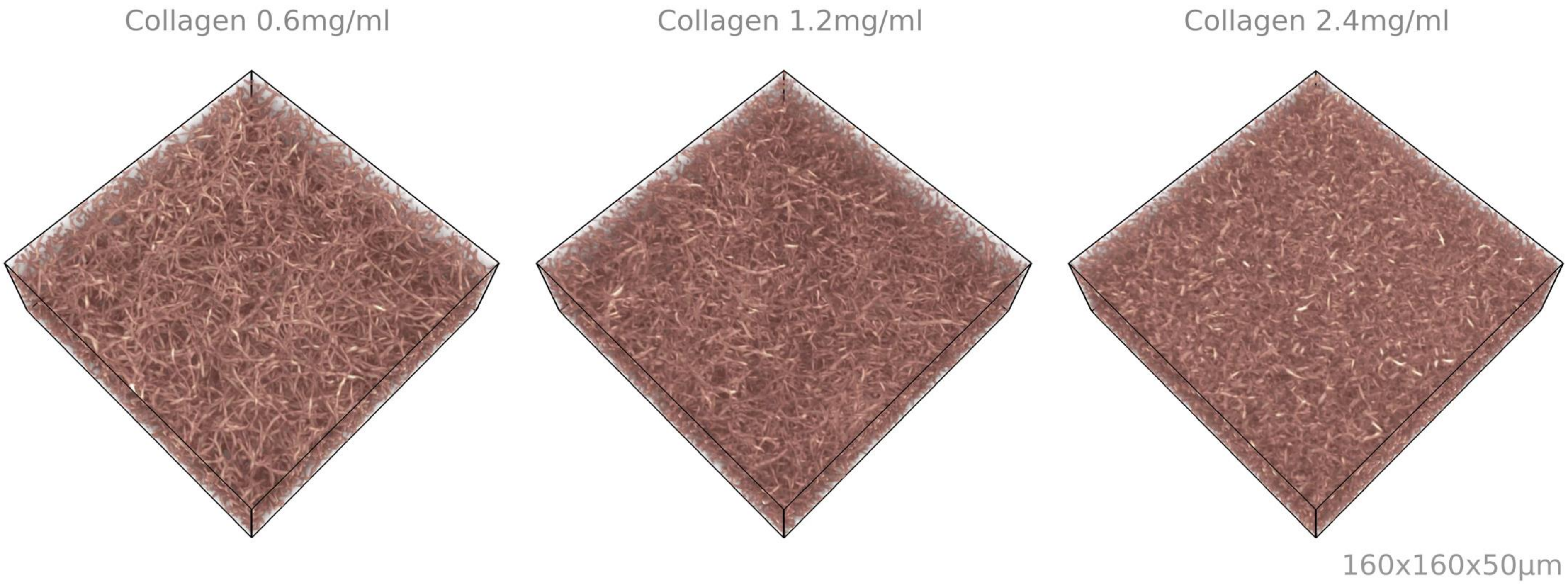
- Methods rely on linear force-displacement response of the substrate
- Natural ECM is highly nonlinear (shear stiffening)
- How to use natural ECM?
  - Micromechanical models with a continuum description
  - On a small spatial scale corresponding to a fiber segment, the local deformation of the fiber segment does not follow the bulk deformation
  - Non-affine behavior caused by
    - Fiber buckling, straightening, or stretching
  - Deformations become affine for a sufficiently large volume of material
  - Averaging the force contributions of all fibers

$$w''(\lambda) = \kappa_0 \begin{cases} e^{\lambda / d_0} & \forall \lambda < 0 \\ 1 & \forall 0 < \lambda < \lambda_s \\ e^{(\lambda - \lambda_s)/d_s} & \forall \lambda_s < \lambda \end{cases}$$

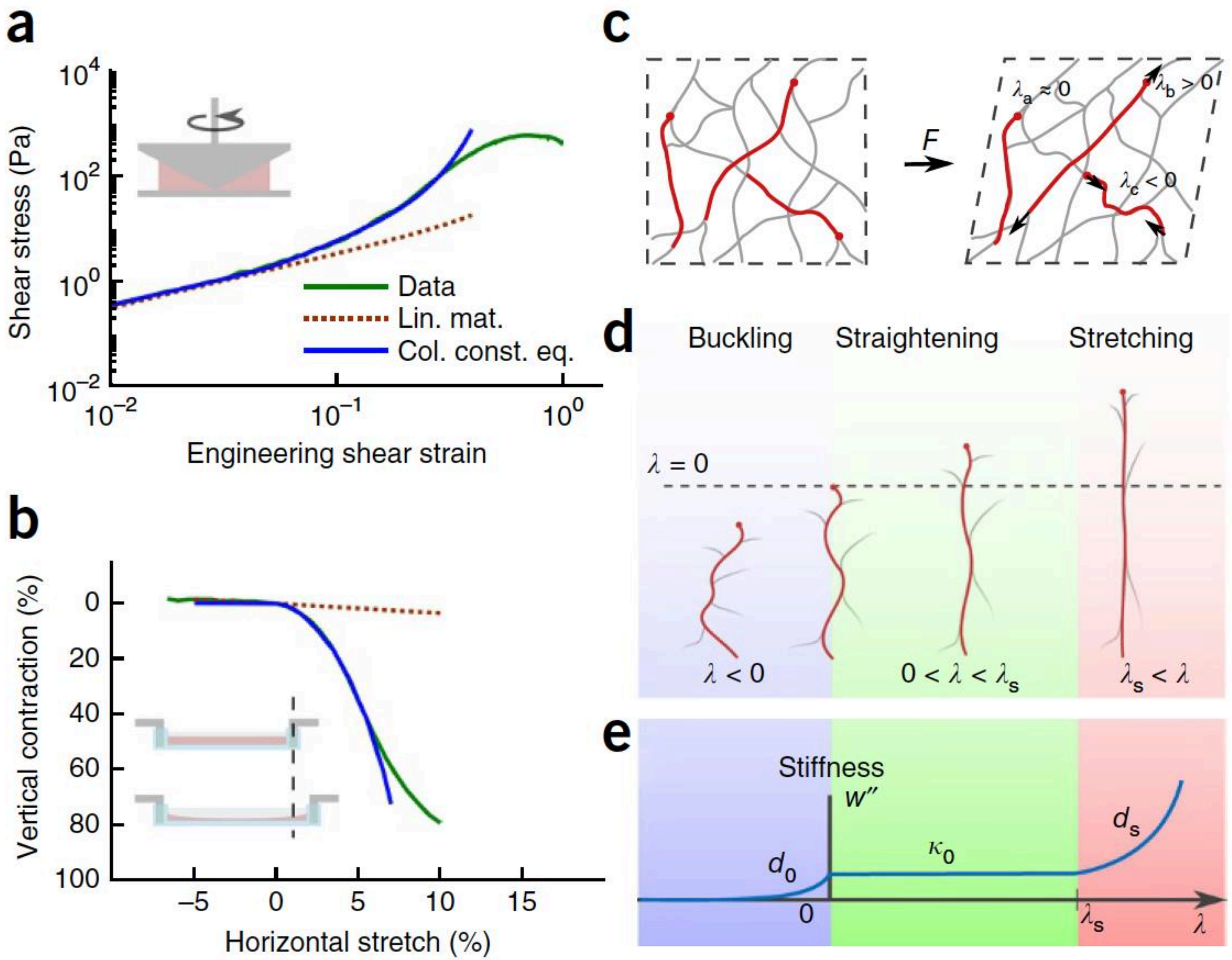
Buckling coefficient

Strain stiffening

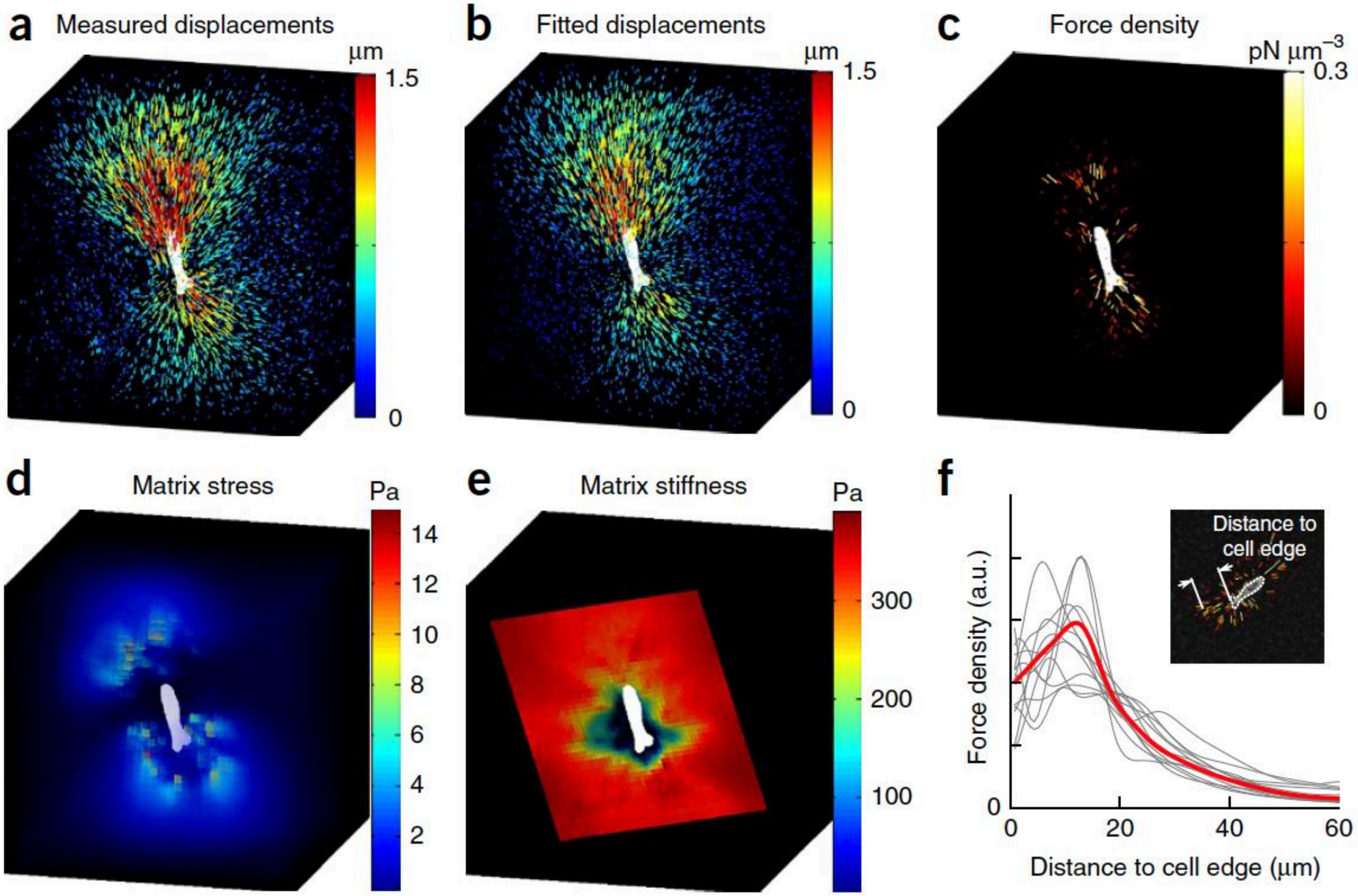
Traction force microscopy: collagen gel



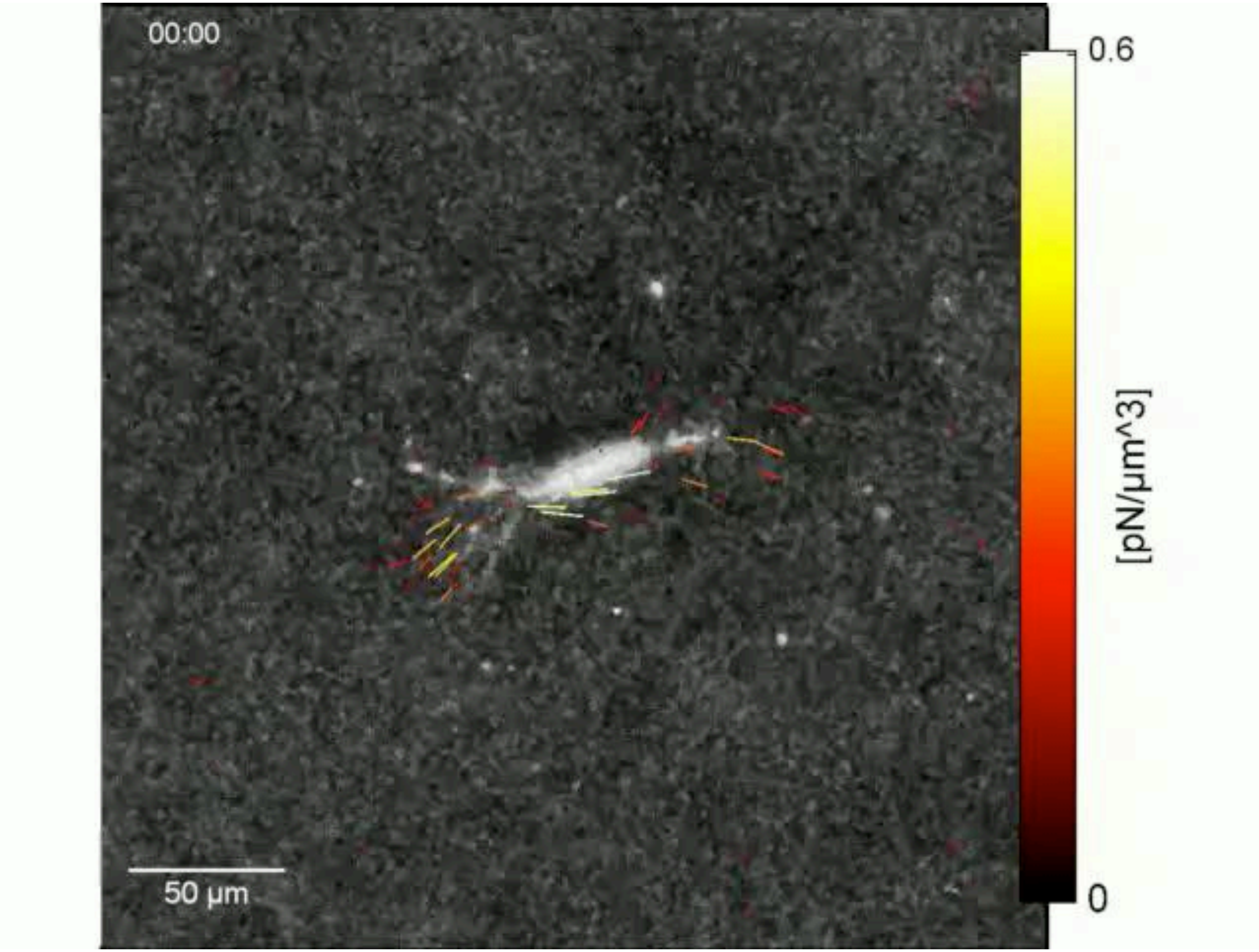
Traction force microscopy: collagen gel



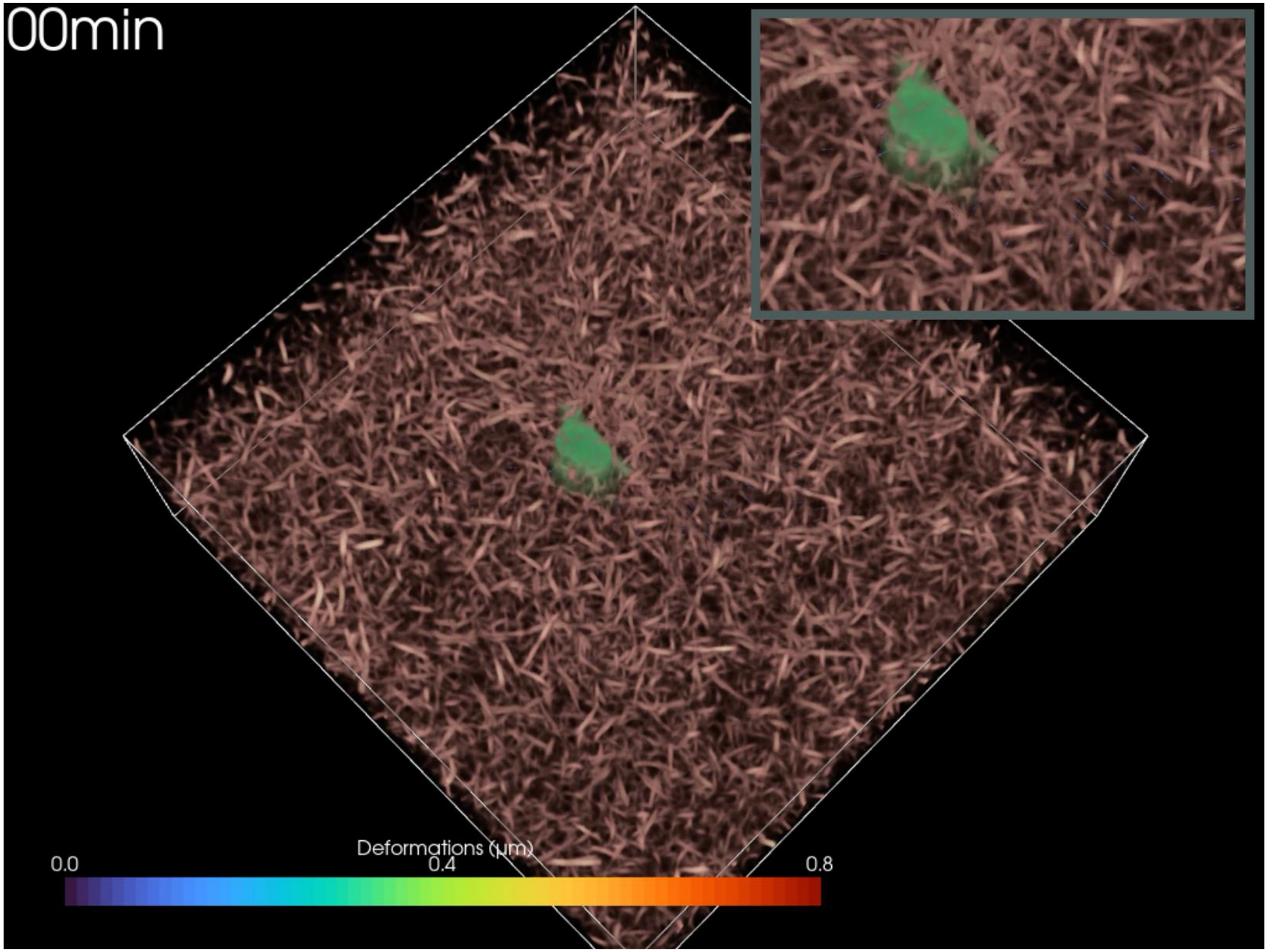
Traction force microscopy: collagen gel

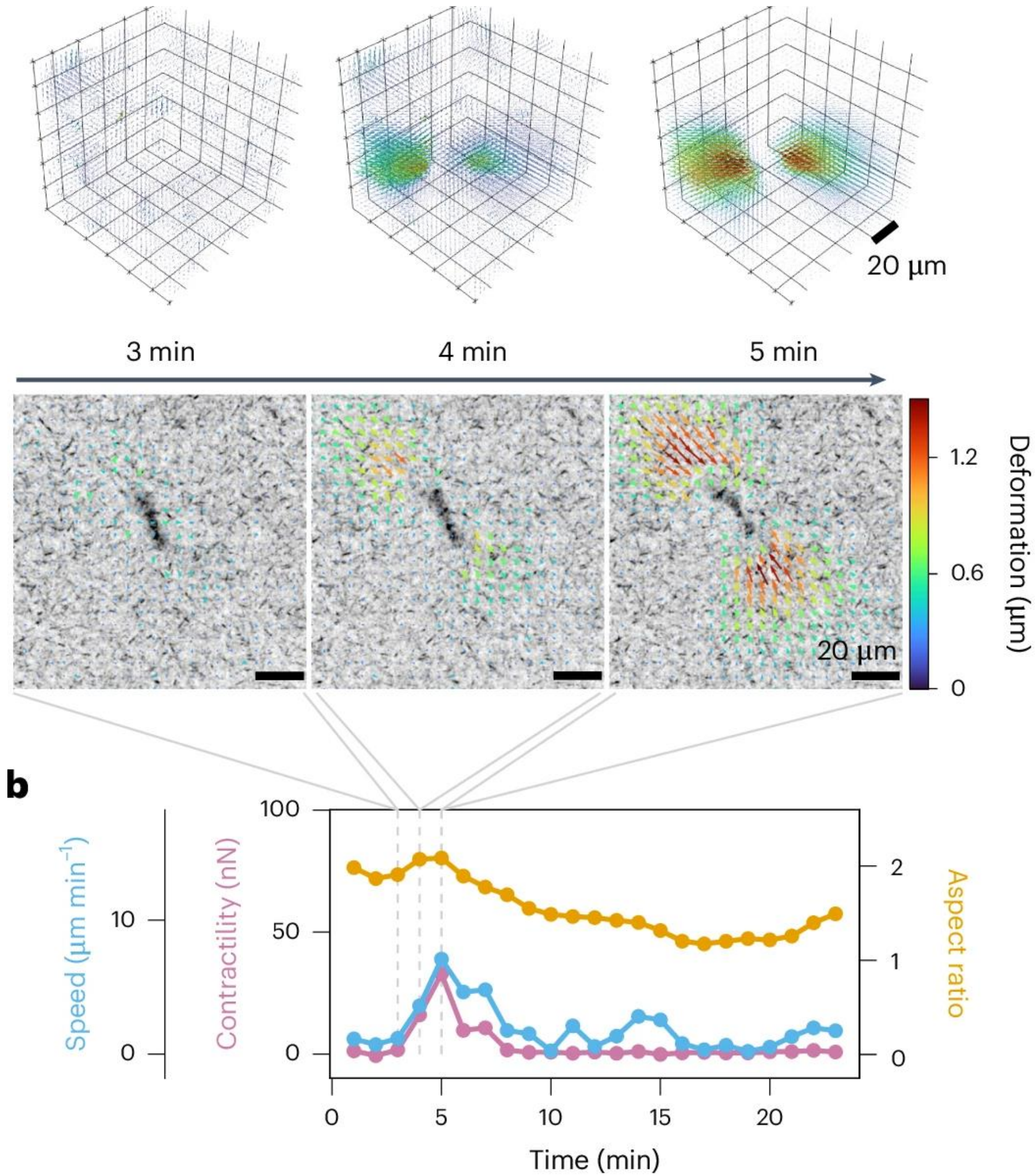


Traction force microscopy: collagen gel



Traction force microscopy: collagen gel

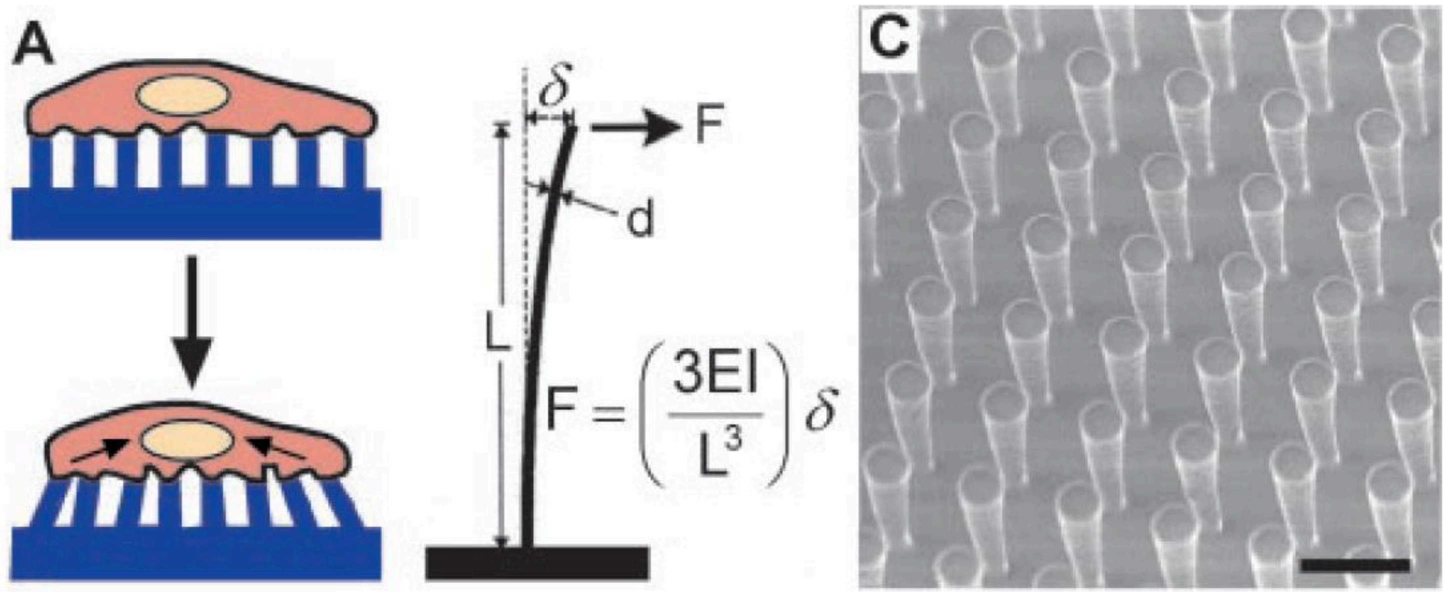




20 µm
3 min
4 min
5 min
20 µm
Deformation (µm)
1.2
0.6
0
b
Speed (µm min^{-1})
10
0
Contractility (nN)
100
50
0
Aspect ratio
2
1
0
0
5
10
15
20
Time (min)

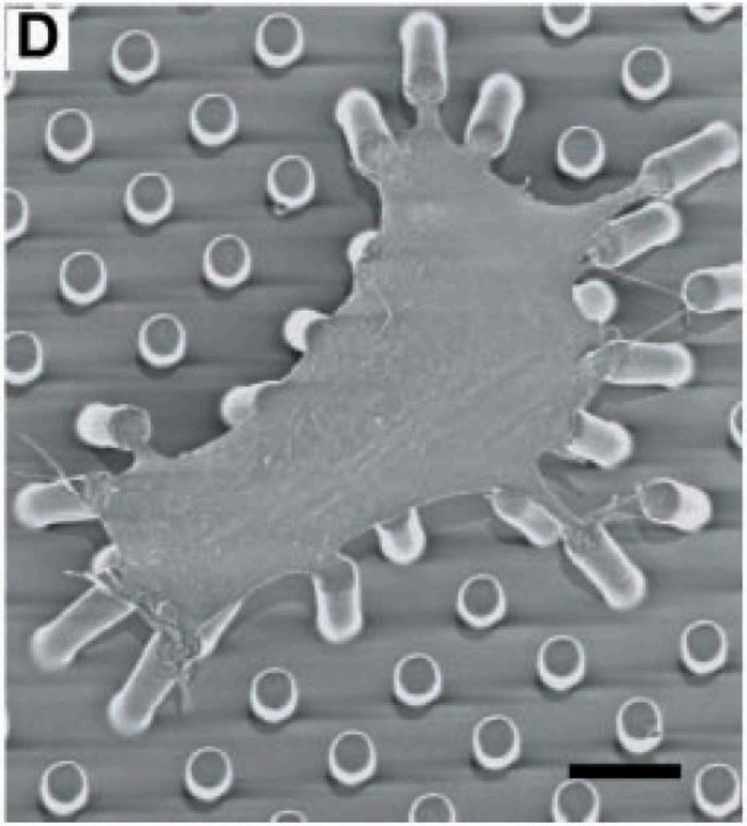
Traction force microscopy: micropillars

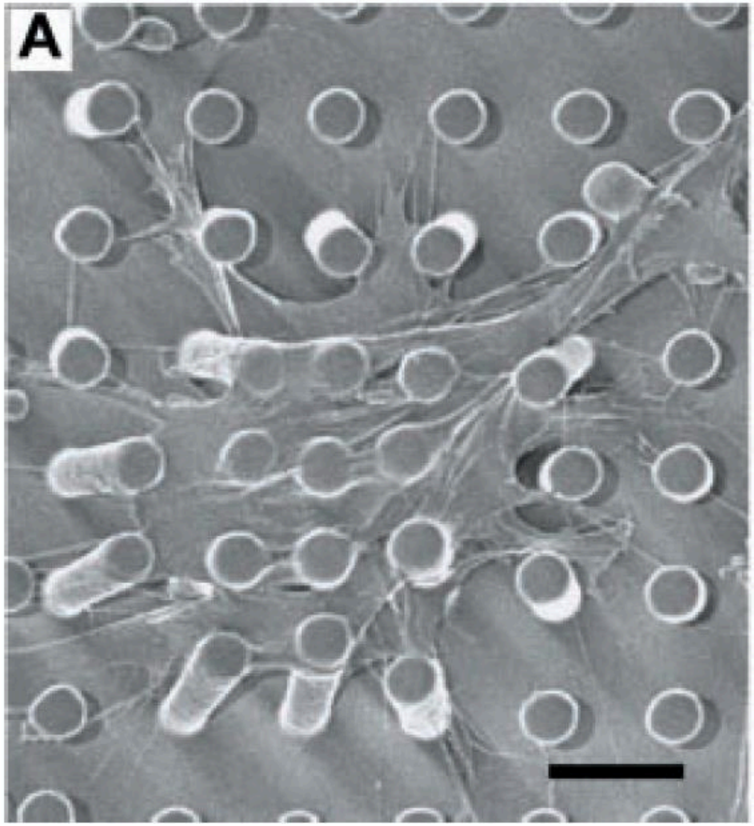
- Microfabricated silicone elastomer pillars
- Small deflections: linear beam theory
- Change geometry to tune pillar stiffness



Traction force microscopy: micropillars

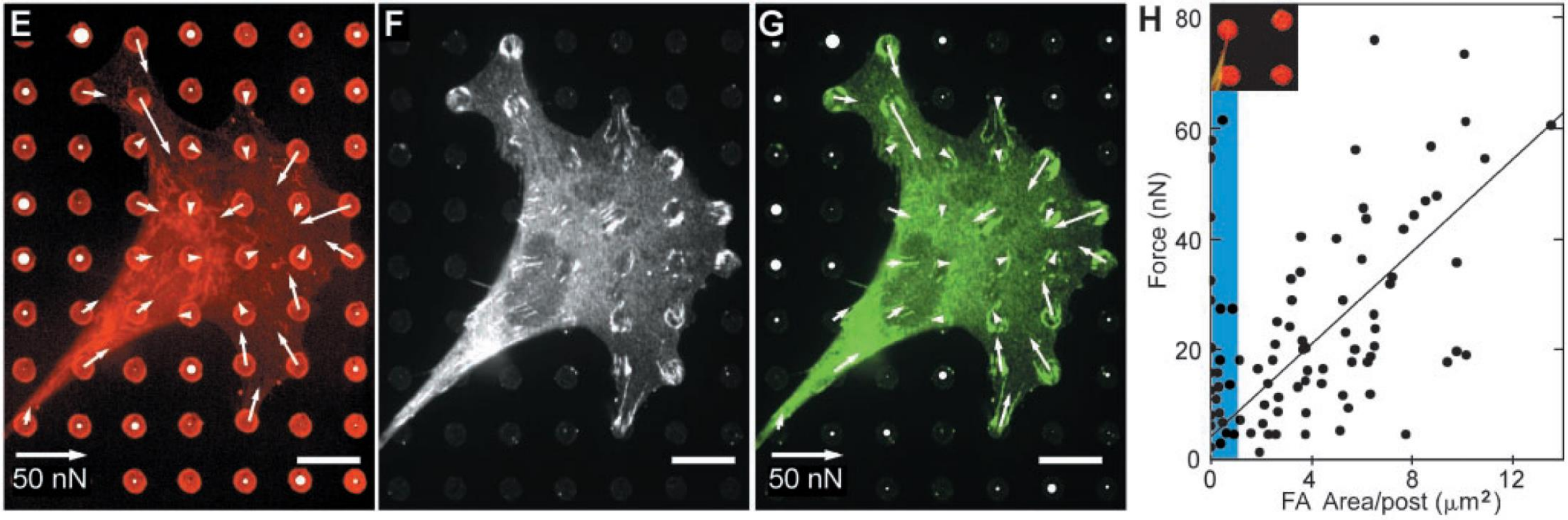
- Cell culture
- Protein coating for cell adhesion
- Scanning electron microscope images





Traction force microscopy: micropillars

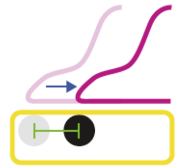
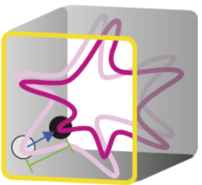
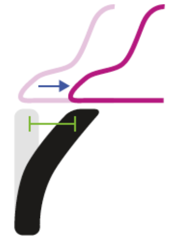
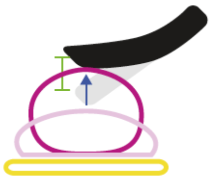
- Measurement of contractile forces
- Fibronectin (E), Vinculin (F and G)



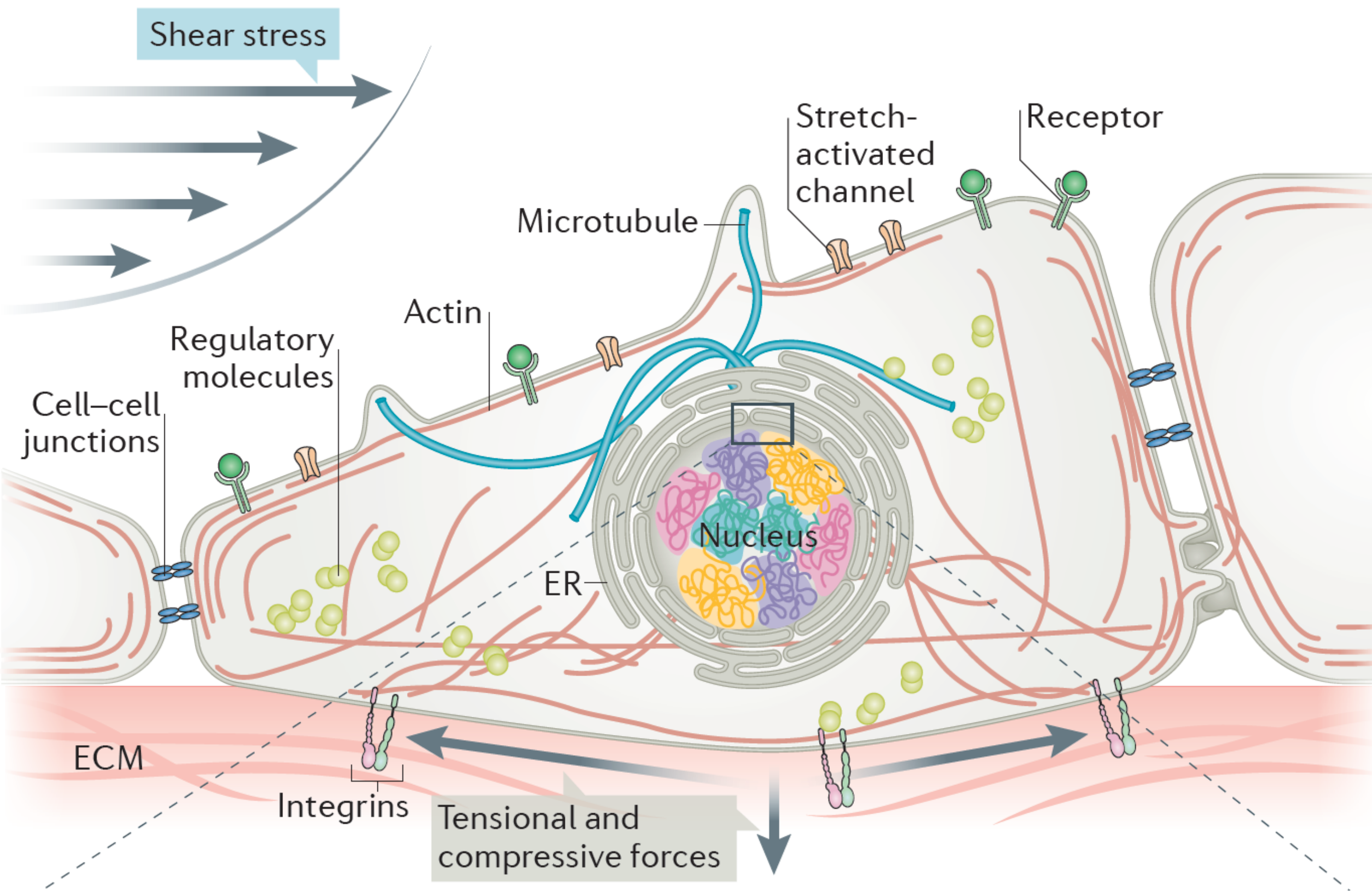
Methods for measuring cellular forces

	Force and stress range	Cells per measurement	Spatial resolution[a]	Substrate and stiffness	Special requirements	Strengths	Major limitations
Collagen gel	N/A	1×10^4 to 1×10^6	N/A	3D collagen type I Young's modulus: 0.01–0.1 kPa	None	Ease of implementation	Qualitative Cannot determine forces from single cells
Tissue pillars	1 µN–0.5 mN 0.02–2.5 kPa	100 to 2×10^6	4 mm	3D collagen type I, Matrigel, or fibrin with embedded PDMS pillars Pillar stiffness: 0.05–1.125 µN µm^{-1}	Tissues <10 mm require microfabrication	High throughput Ease of computation	Requires highly contractile cells Cannot determine forces generated by single cells
TFM	2–120 nN 0.05–0.6 kPa	1 to 1×10^3	2 µm	2D collagen type I; fibronectin; or arginine–glycine–aspartic acid (RGD)-coated PEG, PDMS, or PA Young's modulus: 1.2–1,000 kPa	Hydrogel or PDMS synthesis and functionalization Microparticle tracking algorithms	Uses standard lab equipment and fluorescence microscopy	2D substrates Synthetic substrates with limited biological relevance Computationally expensive Requires cell lysis or manipulation
Micropillar	50 pN–100 nN 0.06–8 kPa	1–10	1 µm	2D collagen type I, collagen type IV, or fibronectin-coated PDMS Pillar stiffness: 1.9–1,556 nN µm^{-1}	Microfabrication PDMS functionalization	Ease of implementation and computation	Forces are independent for posts Fabrication
3D TFM	Not characterized 0.1–5 kPa	1	5 µm	3D RGD-conjugated PEG Young's modulus: 0.6–1 kPa	Confocal microscopy 3D mesh editing and finite-element software 3D, MMP-cleavable synthetic hydrogels	Fully resolved 3D tractions in physiologic 3D environments	Currently limited to single cells Computationally expensive
DNA hairpin	4.7 pN–2 nN 0.15–50 kPa	1	0.2 µm	2D RGD-conjugated DNA hairpin on glass Young's modulus: 50 GPa	DNA hairpin synthesis	High resolution with standard fluorescence microscopy	2D Currently limited to glass substrates Long sample-prep time

Methods for measuring cellular forces

	Force range	Length scale	Measured quantity	*In vivo?*	Strengths	Limitations	References	Schematic
2D traction microscopy	1–10^4 Pa	10^{-1}–10^3 µm	Substrate displacement	No	Absolute measurement Tunability of substrate stiffness Output is a 2D map	Computationally involved High sensitivity to displacement noise	21–42	
3D traction microscopy	10–10^4 Pa	10^{-1}–10^2 µm	ECM displacement	No	Cells in 3D environment Output is a 3D map	Computationally very involved Unknown ECM material properties close to the cell Physiological ECM is non-linear	38,39	
Micropillars	10^{-2}–10^2 nN	10^{-1}–1 µm	Pillar displacement	No	Absolute measurement No reference image required Simple force calculation	Discrete rather than continuous adhesion Difficult to compare to physiological environments Small stiffness range	44–54	
Cantilevers	10^{-2}–10^2 nN	10–10^3 µm	Cantilever displacement	No	No reference image required Simple and precise force measurements in real time	Requires contact Low throughput	56–59	

Nuclear mechanobiology



Nuclear mechanobiology

- Forces applied to the nucleus through cytoskeleton
  - Modulate the effect of cytoplasmic signals
  - Directly trigger changes in gene expression

- Why involve the nucleus in mechanosensing?
  - Distinguish between small force that only affect the cell surface and larger forces that deform the nucleus

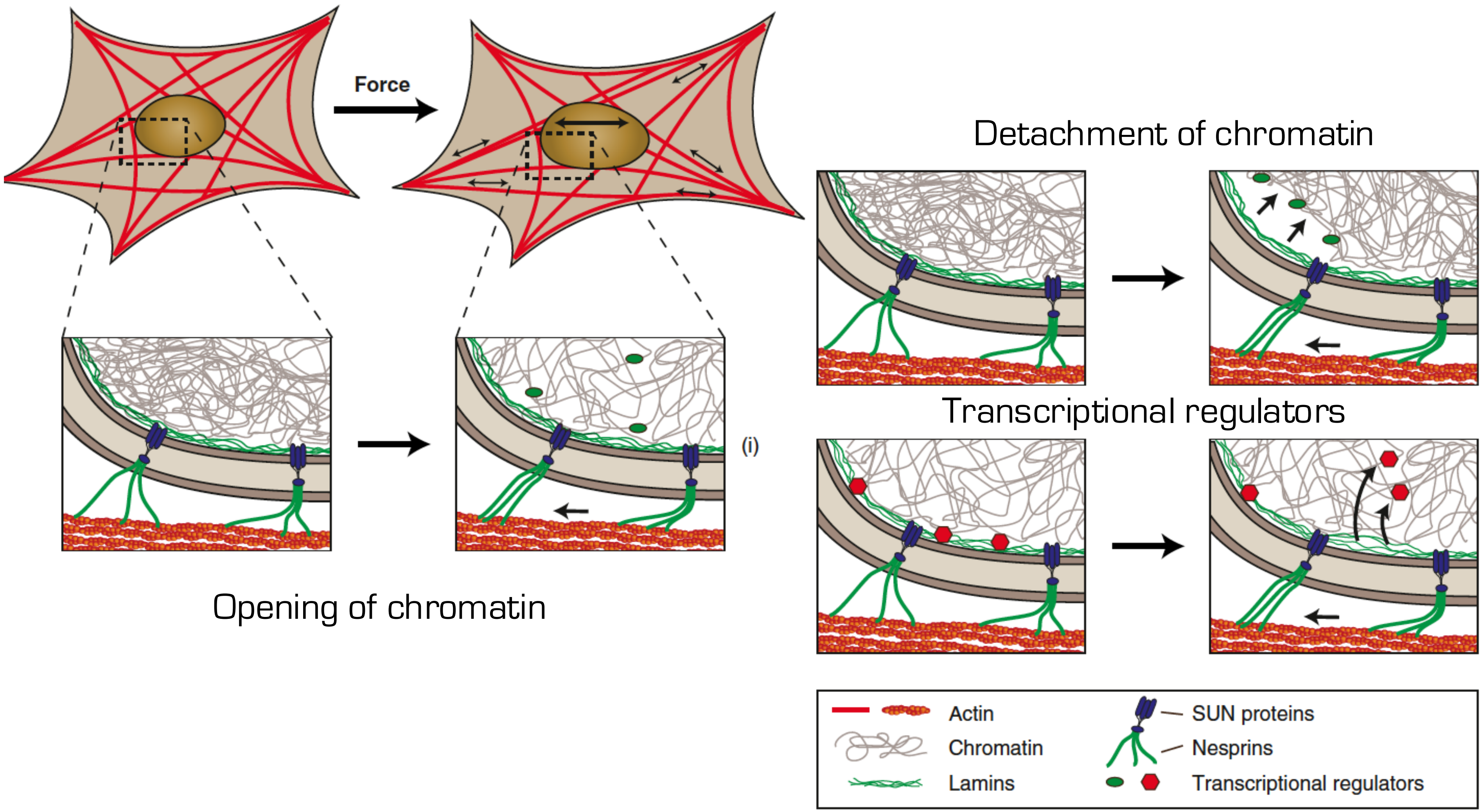
Nuclear mechanobiology

- Adapting the mechanical properties of the nucleus for genetic control
    - Interplay of cytoskeleton-nucleus links
    - Integrity and composition of the nuclear lamina
    - The degree of DNA packaging into chromatin

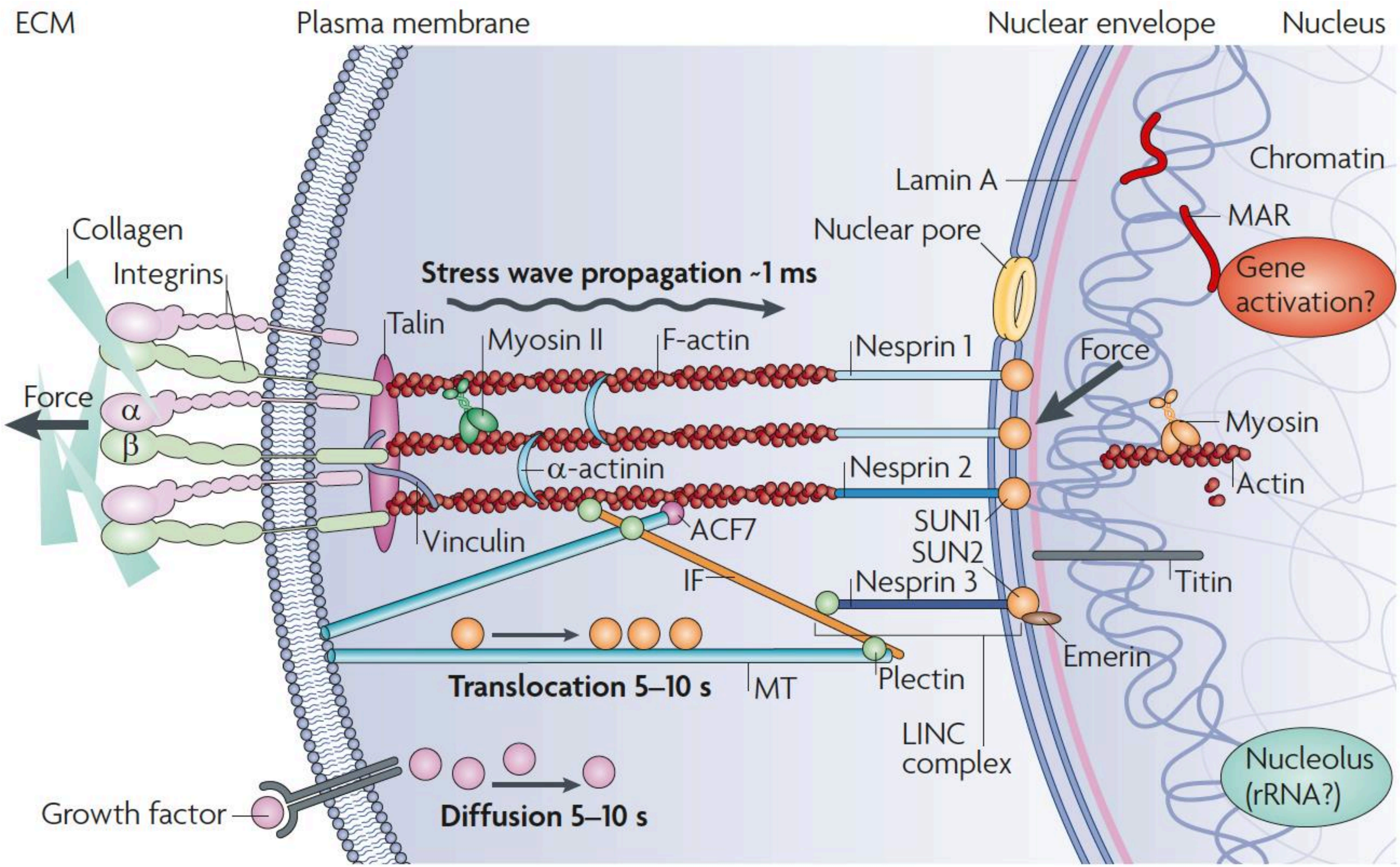
- Changes in the morphology and and deformability of the nucleus
- Changes in the chromatin organization

- Cytoskeleton: Bridge between the cell membrane and the nucleus
    - Linker of nucleoskeleton and cytoskeleton (LINC) complex
    - Direct transmission of mechanical signals

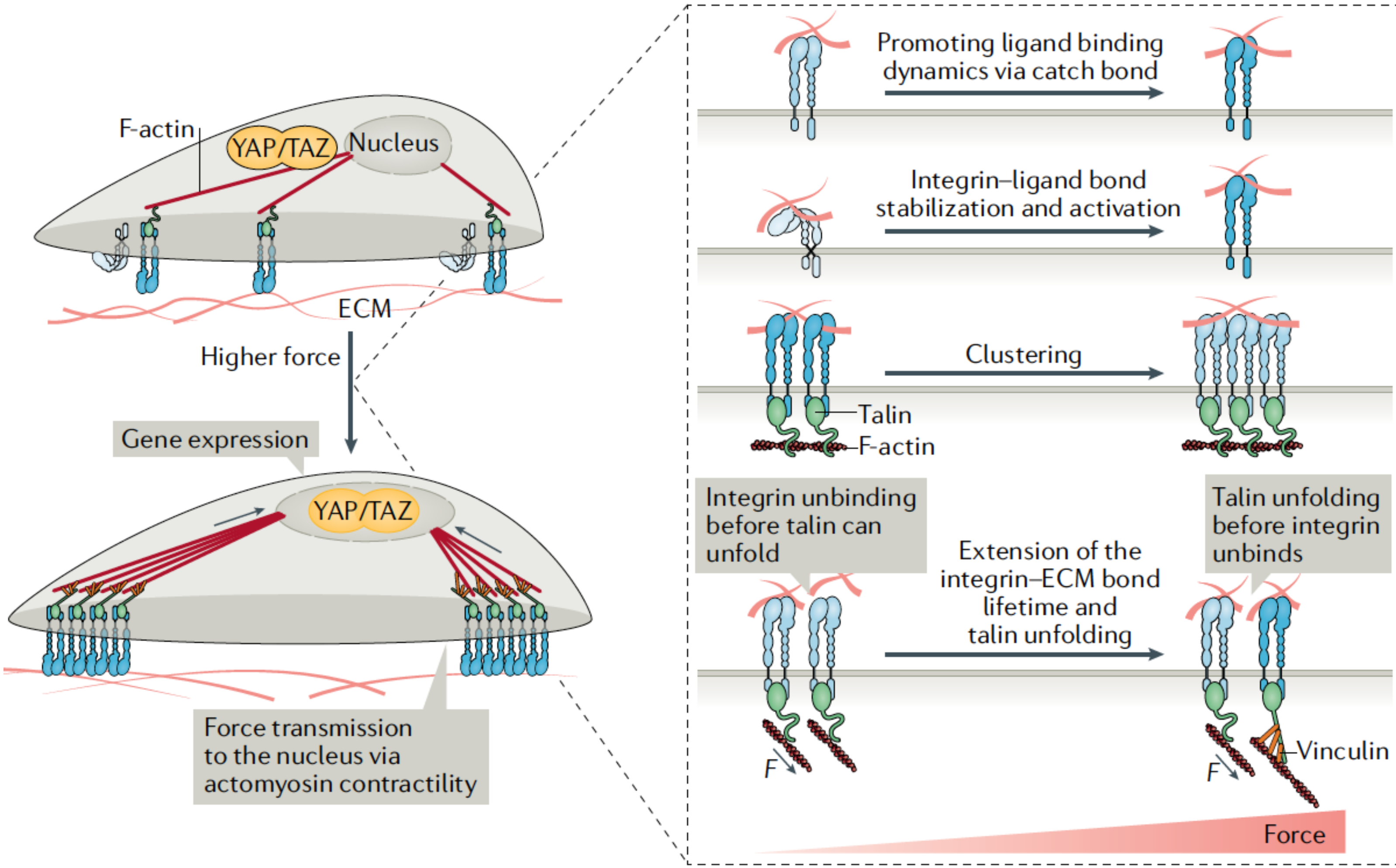
Potential mechanisms of nuclear mechanosensing



From integrin to the nucleus



From integrin to the nucleus



From integrin to the nucleus