Cell adhesion & Mechanics

Lecture 1

Benoit Ladoux

Outline

1 Large-scale mechanical responses of single cell responses in response to external cues

Single cell responses to substrate stiffness and adhesive cues

Mukund Gupta
Jimmy le Digabel
Léa Trichet
Bibhu Sarangi
Leyla Kocgozlu
Raphael Voituriez (UPMC)

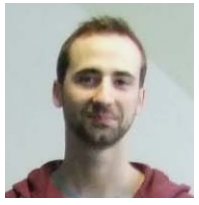





René-Marc Mège
Charlotte Plestant
Pierre-Olivier Strale
Rima Seddiki
Emmanuelle N'Guyen

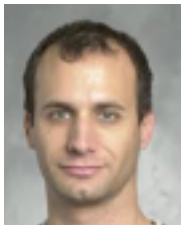

2 Large-scale coordinated movements during collective cell migration


SRK Vedula CT. Lim



G. Peyret
Thuan Beng Saw
Shreyansh Jain

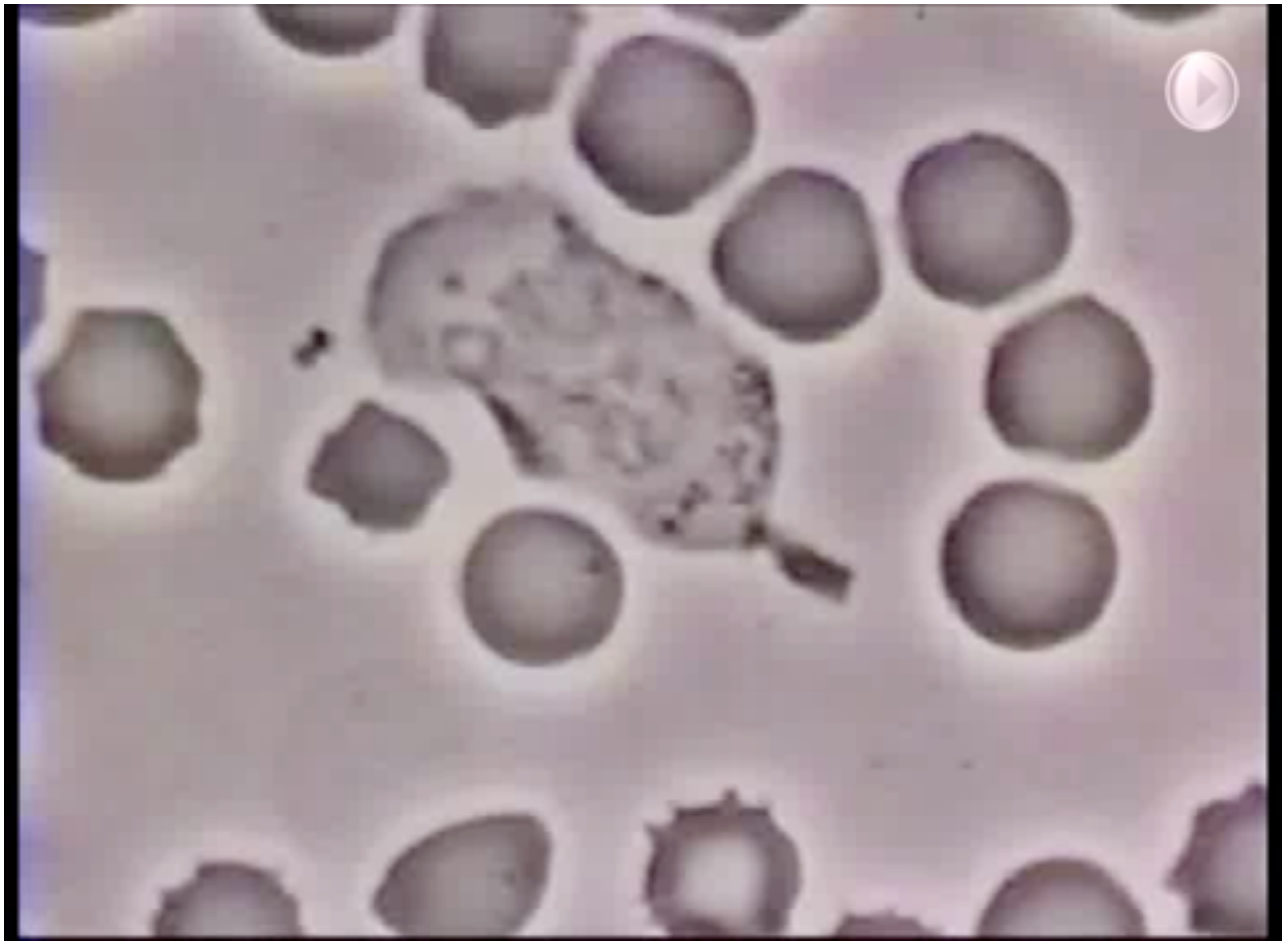





WJ Nelson AJ. Kabla N. Gov X. Trepat

TEASER

Motility



Neutrophil chasing a bacteria in the middle of red blood cells

Motility: Amoeba proteus

Figure 2-1 *Amoeba proteus* feeding. The slow random crawling of *A. proteus* is seen in this sequence of photographs taken at 20-second intervals. Although it seems chaotic, *A. proteus* changes its movements when it senses food. A rapidly swimming protozoan that by chance blunders into the amoeba (see arrow at 100 seconds) causes the latter to commence a slow circling movement. In this case the prey escaped, but not all cells are so lucky.

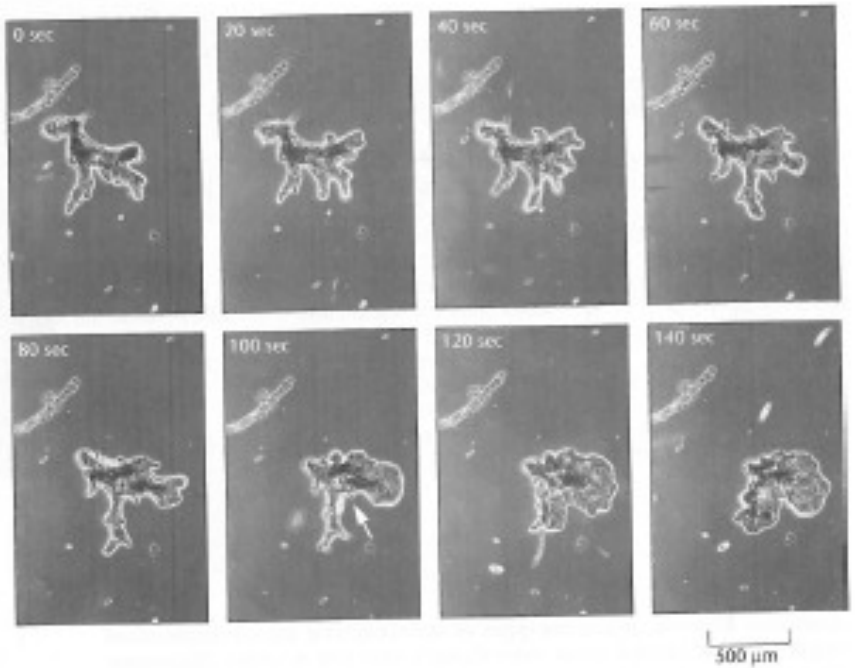
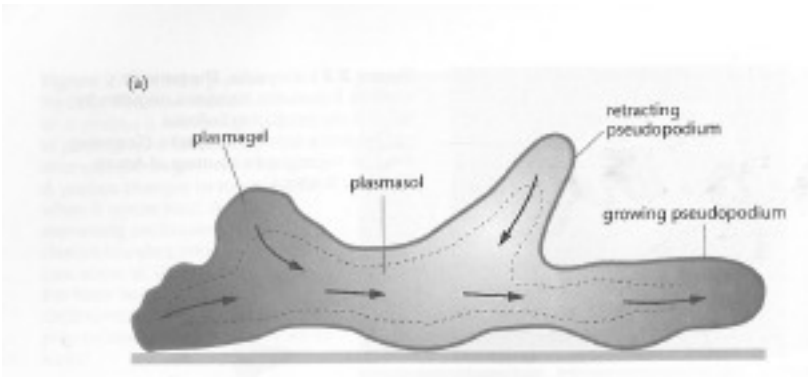
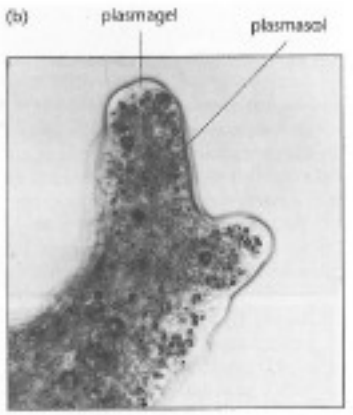


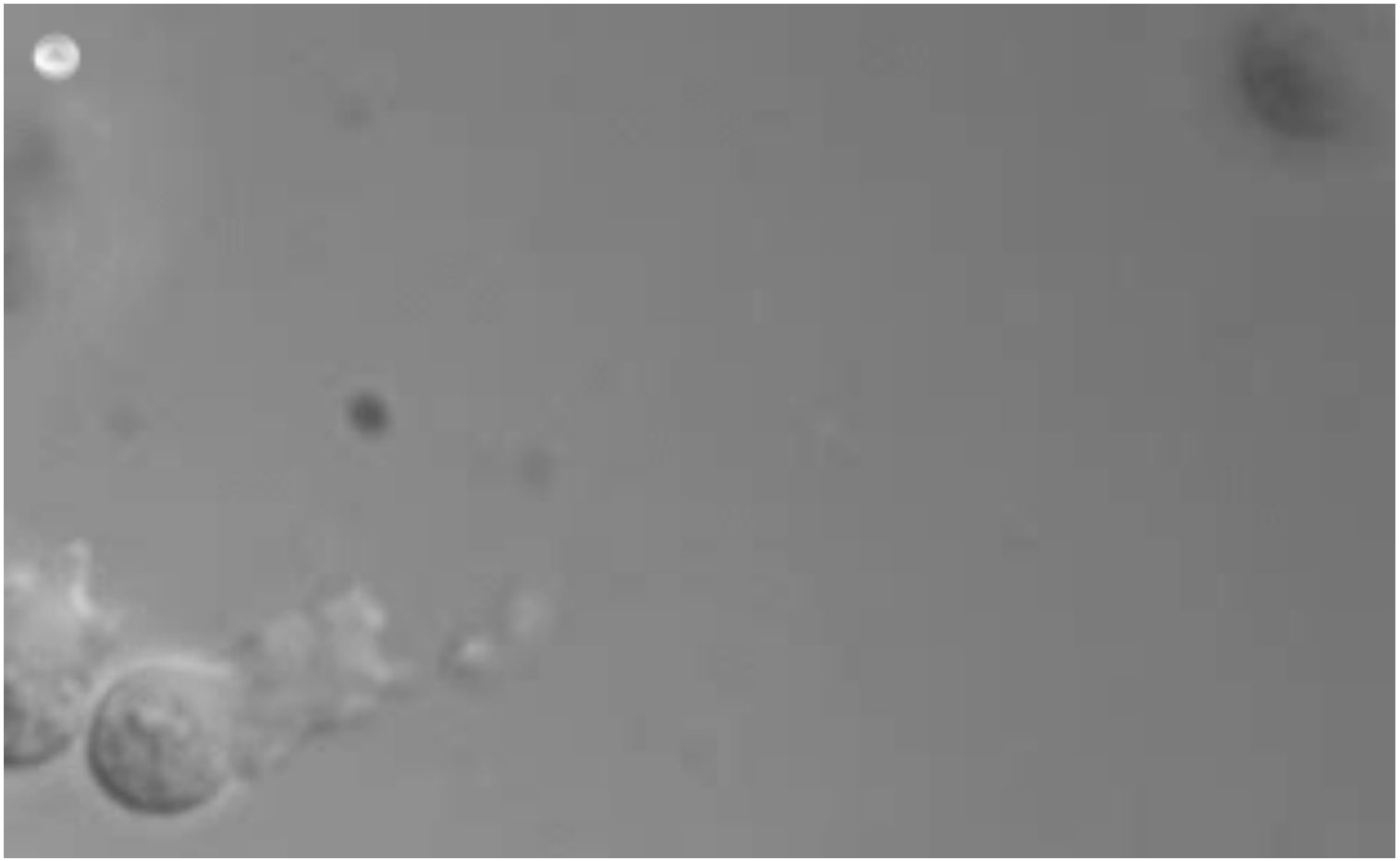
Figure 2-4 Cytoplasmic streaming during the migration of *Amoeba proteus*. (a) The central region of more fluid cytoplasm, or plasmasol, flows into pseudopodia as they form and out again as they retract. The outer shell of more rigid cytoplasm appears to form from the plasmasol at the tip of the growing pseudopodium. (b) Tip of the pseudopodium of *A. proteus*. Note the transparent layer beneath the plasma membrane (plasmagel) that excludes vacuoles, mitochondria, and other organelles.





Cell movements, D. Bray

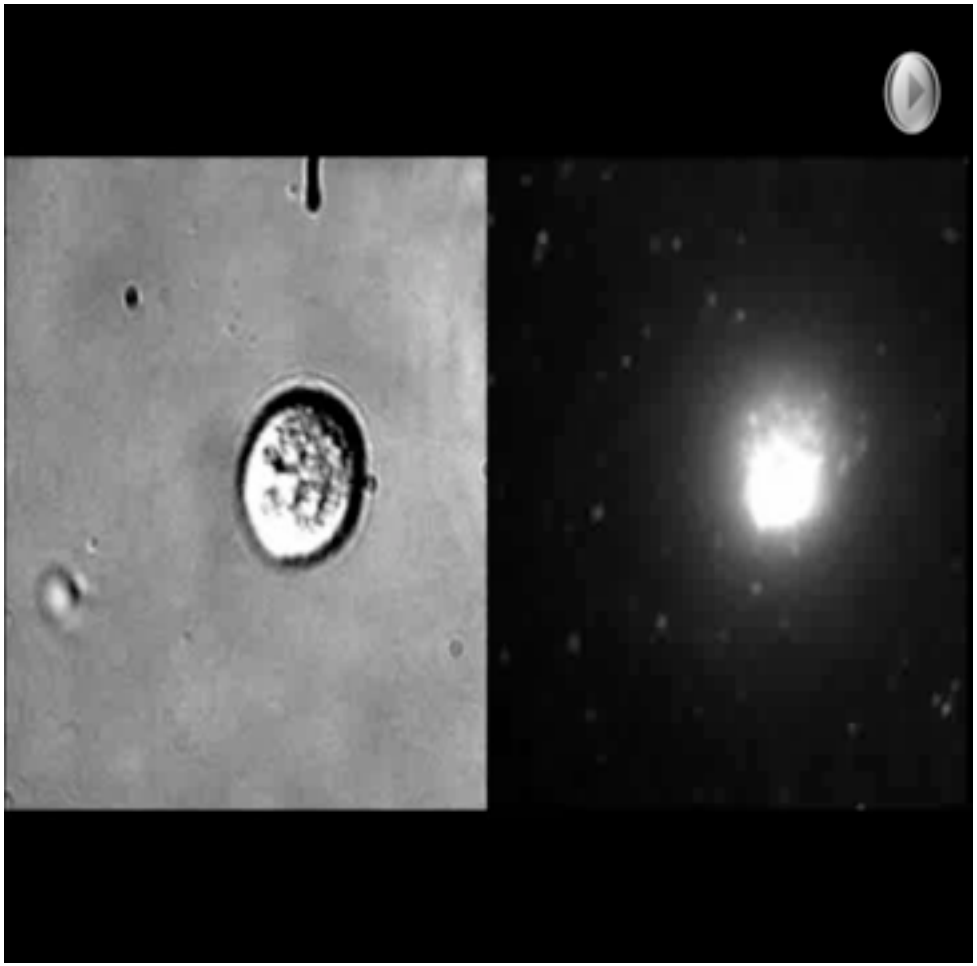
Motility



Worm cell

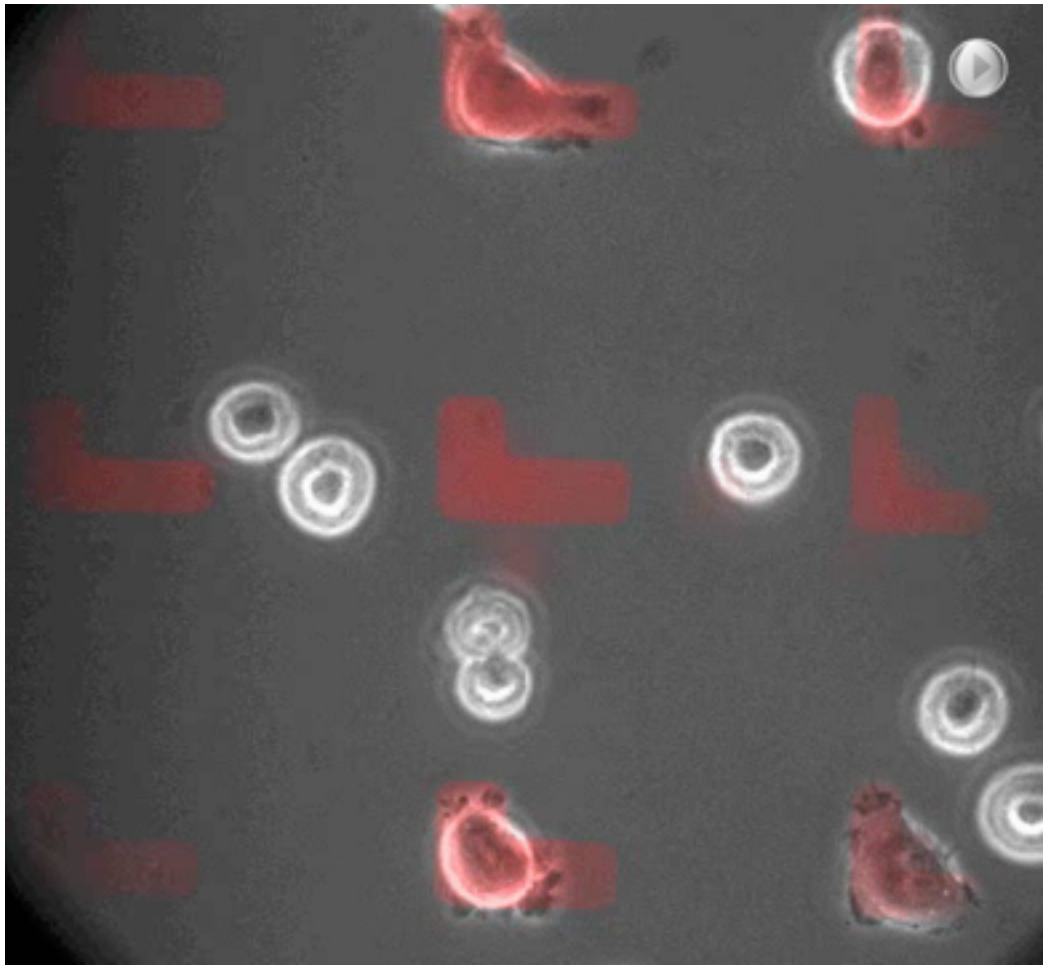
Movie by Julie Plastino, Institut Curie

Division and Adhesion



Fibroblast by DIC (Nomarski)

Specific *versus* non specific interactions



Movie by Manuel Théry, CEA

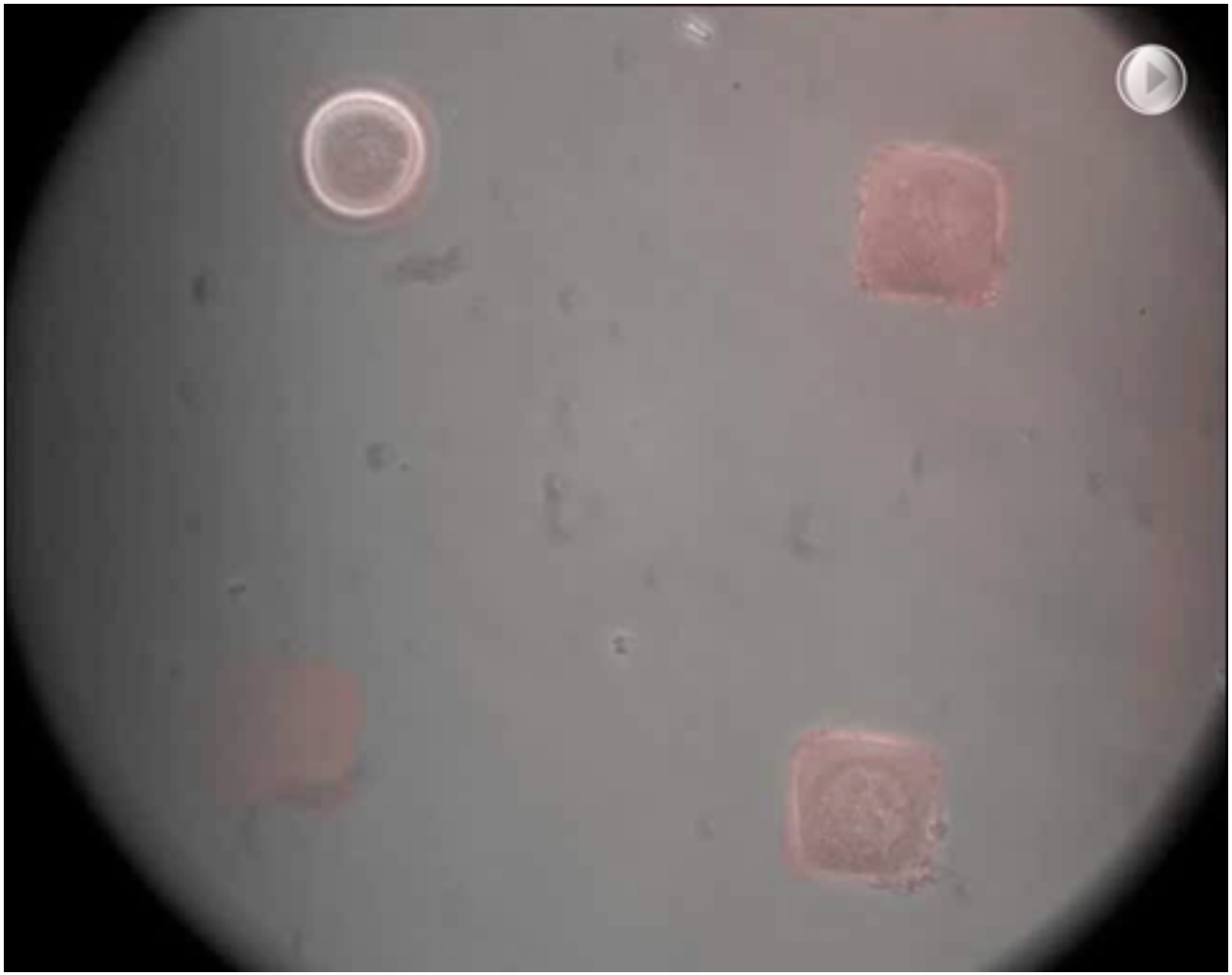
Movie in french : « La vie après la mort d'Henrietta Lacks » by Mathias Théry

Adhesion versus Motility

Macrophage crawling on a surface

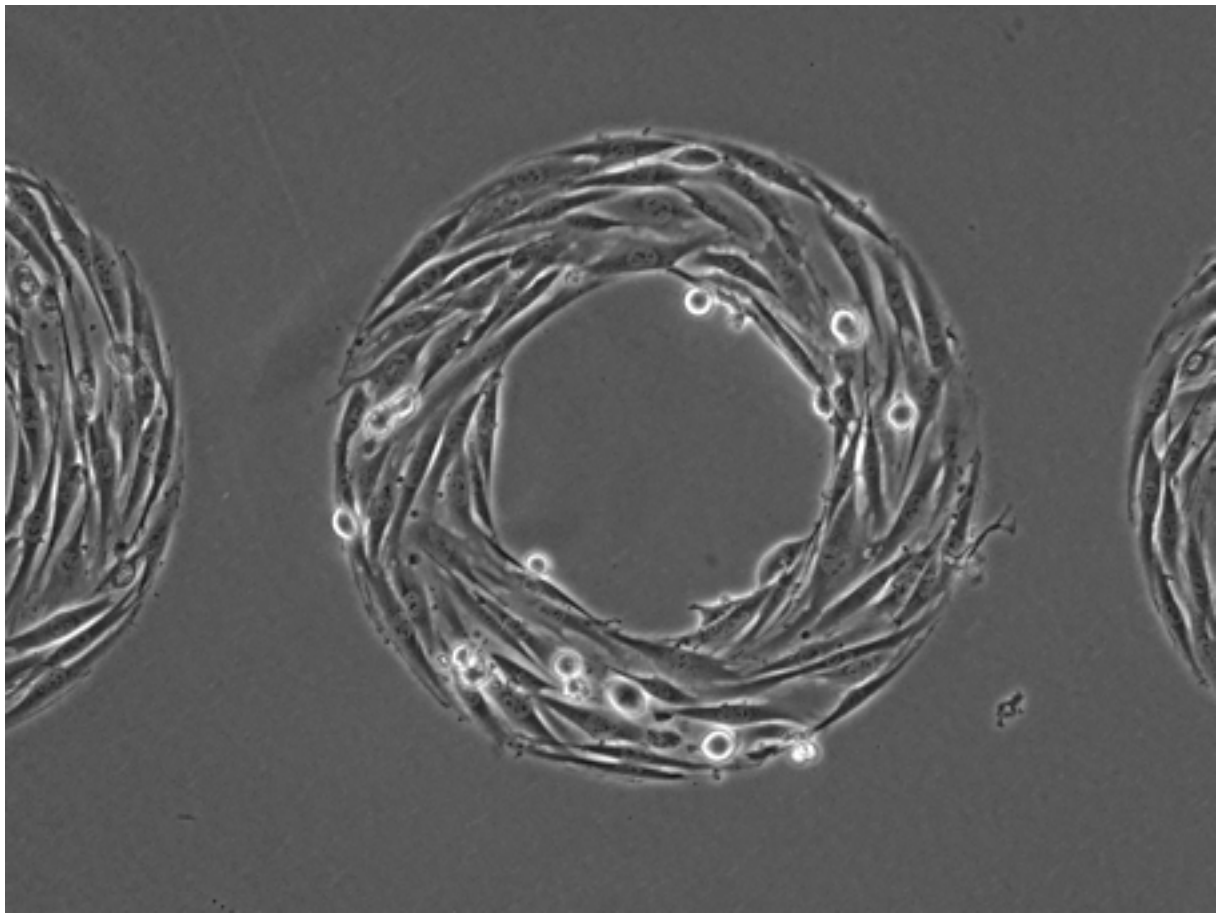
Paul Matsudaira's lab (MIT)

… Complication : Affinity / Lability



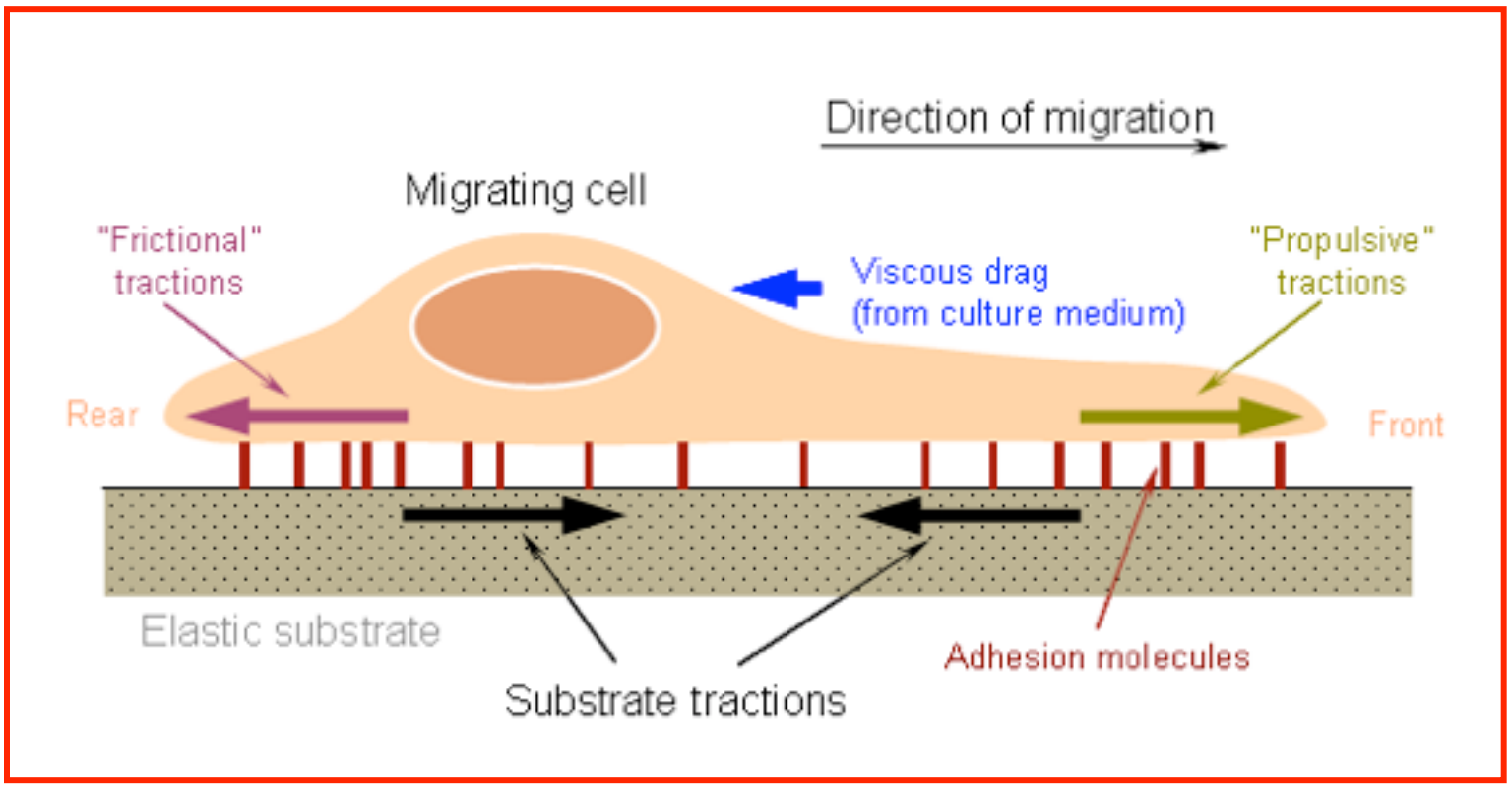
« **Tango** » movie by Manuel Théry, CEA

Collective movements



Shreyansh Jain MBI, Singapore

Cell movement and forces



a

100

10

α_5 Expression

0.17×

0.47×

1×

Mean cell speed (μm h^{-1})

10^{-8}

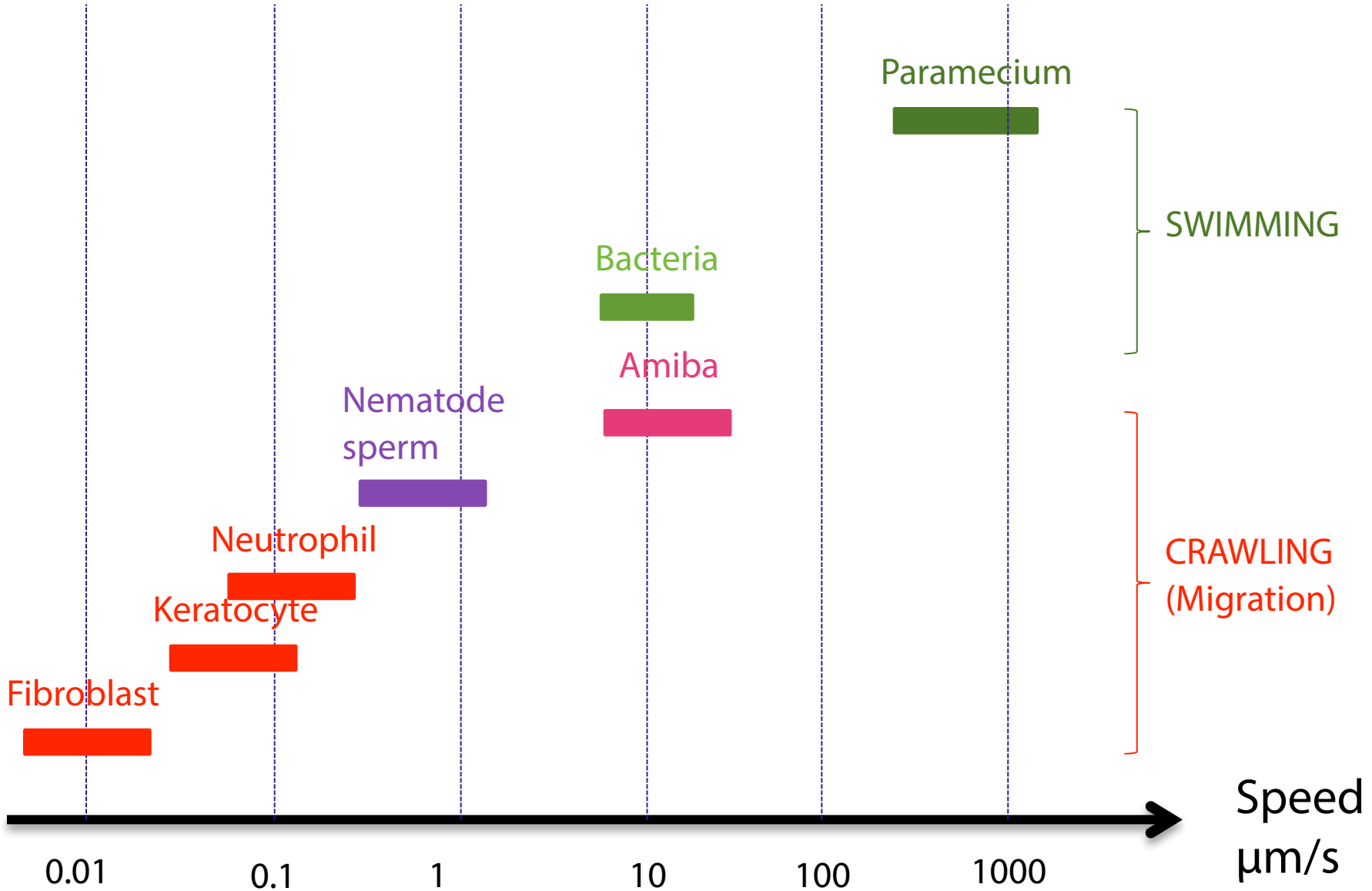
10^{-7}

10^{-6}

Mean detachment force (N)

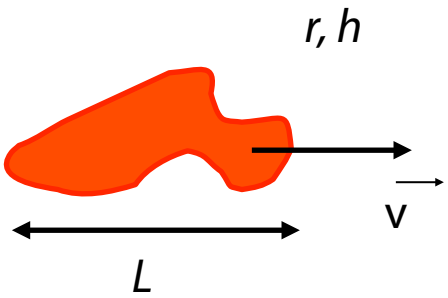
Palecek et al., Nature (1997) 385, 537

Migration speeds



Cell movements

Reynolds number

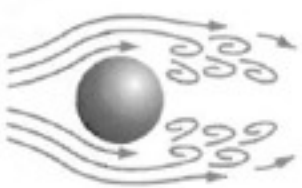


$$\mathrm{Re} = \frac{vL\rho}{\eta} \left(\frac{Inertia}{Viscosity} \right)$$

Figure 1-3 Fluid flow at different Reynolds number (*Re*). At low *Re* the fluid flows smoothly, following the contours of the obstacle, but as *Re* increases small vortices develop. These are at first stationary with respect to the obstacle but at higher *Re* they are shed periodically. At very large *Re* fluid movements downstream of the obstacle become chaotic.

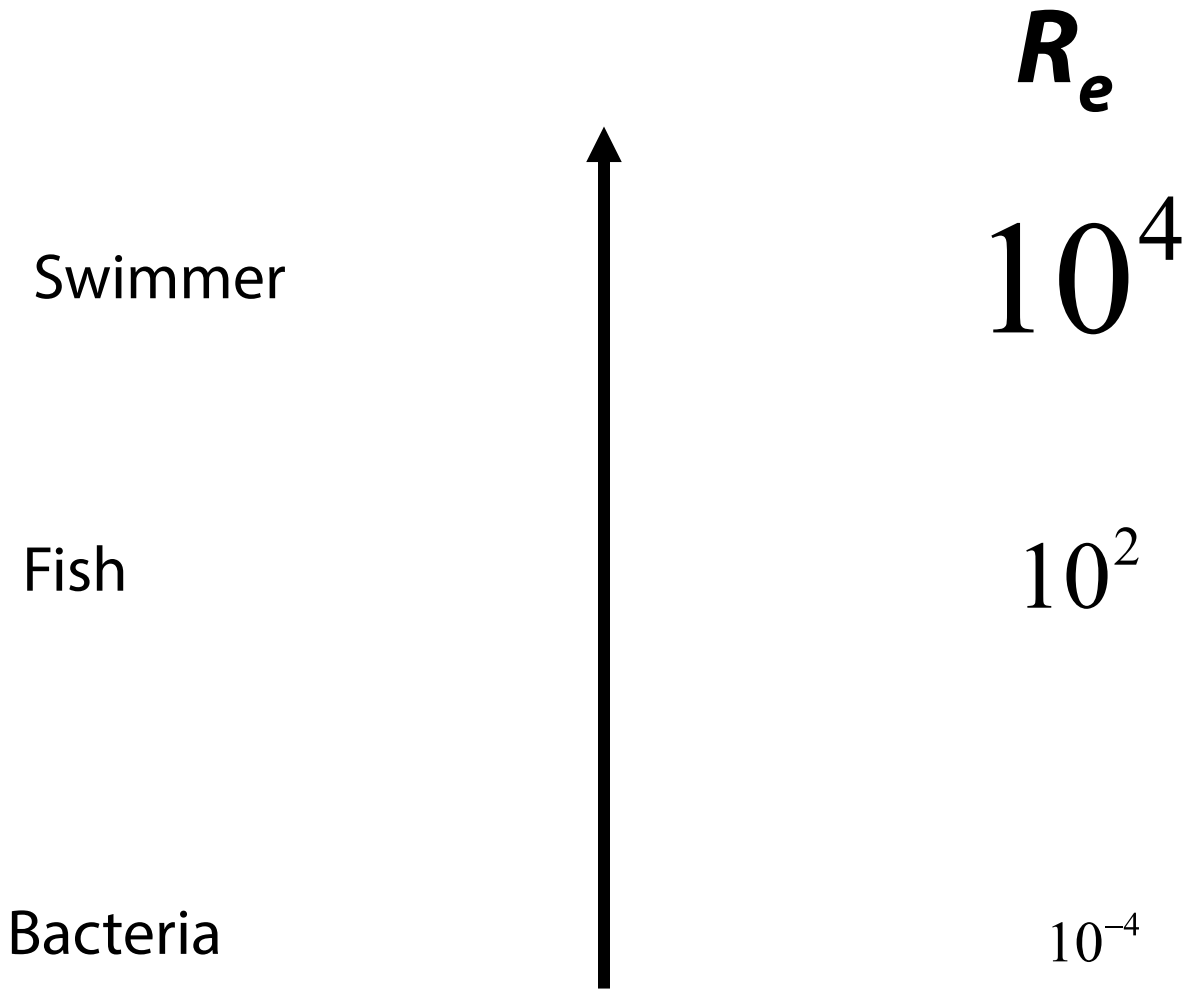
Re < 10
laminar flow

Re 10–40
vortices form and are maintained



Re 40–20,000
vortices form and are periodically shed

Reynolds number



Life at low Reynolds numbers

Navier - Stokes:

$$-\nabla p + \eta \nabla^2 \vec{v} = \rho \frac{\partial \vec{v}}{\partial t} + \rho (\vec{v} \cdot \nabla) \vec{v}$$

If $Q \ll 1$:

Time doesn't matter. The pattern of motion is the same, whether slow or fast, whether forward or backward in time.

The Scallop Theorem

Figure 6

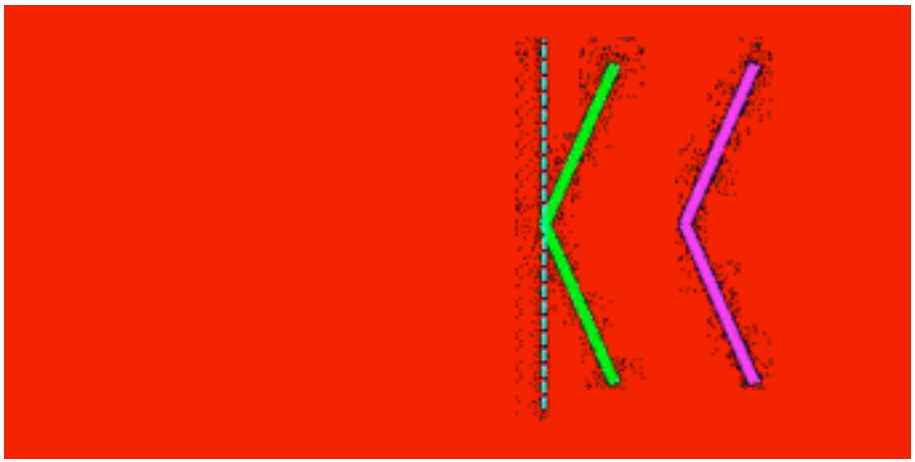
Purcell, 1977

Swimming in highly viscous media

$\Rightarrow$ « Scallop theorem » (Purcell, 1977): in a viscous regime, no reversible movement in time.

Inertia

Viscous



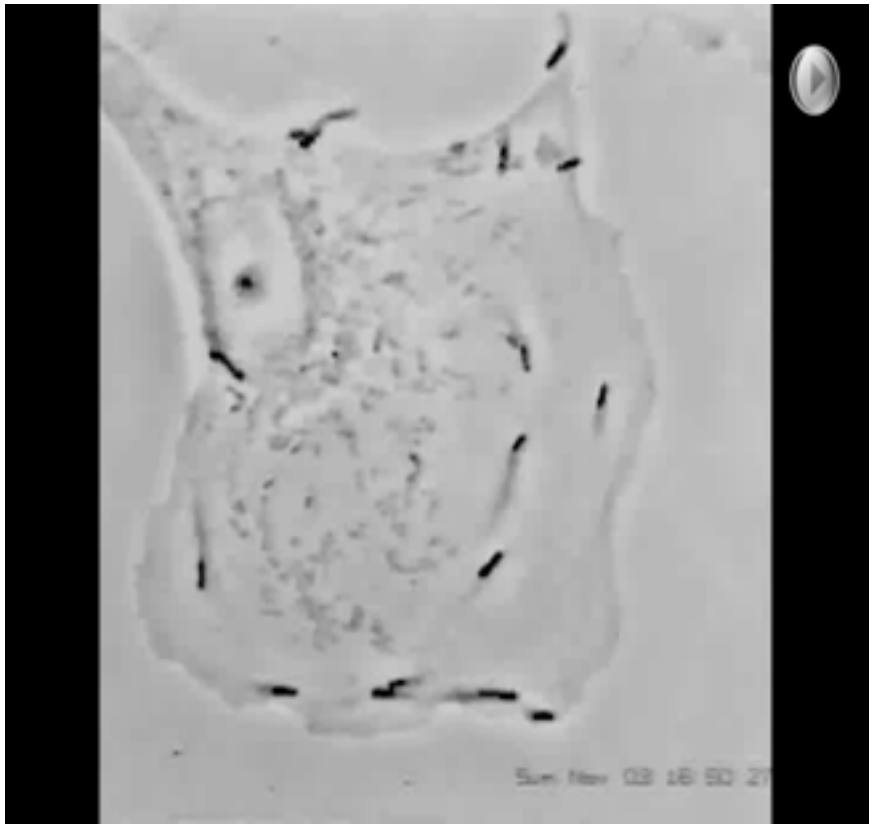
Thèse Rémi Dreyfus, ESPCI

Cellular processes : No inertia so forces are proportional to speed and not acceleration!

Why biophysics?

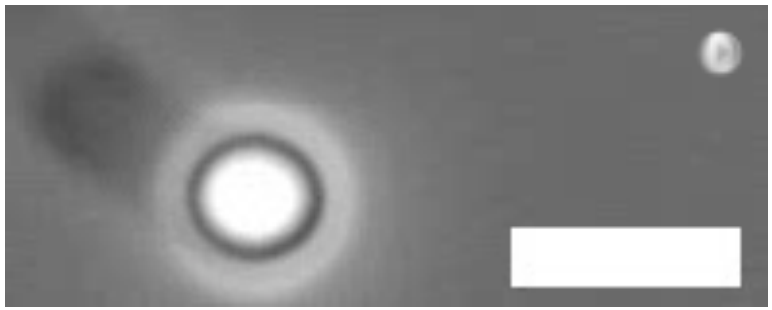
Biomimetic approach

Mouvement de la *Listeria monocytogenes*



***Listeria monocytogenes* moving in PtK2 cells**
These pathogenic bacteria grow directly in the host cell cytoplasm. The phase-dense streaks behind the bacteria are the actin-rich comet tails. Actin-based motility is also used in cellular motility; this cell is using it's cytoskeleton to crawl toward the lower right-hand corner.
Accelerated 150X.
--Julie Theriot & Dan Portnoy

Rocketting oil droplets

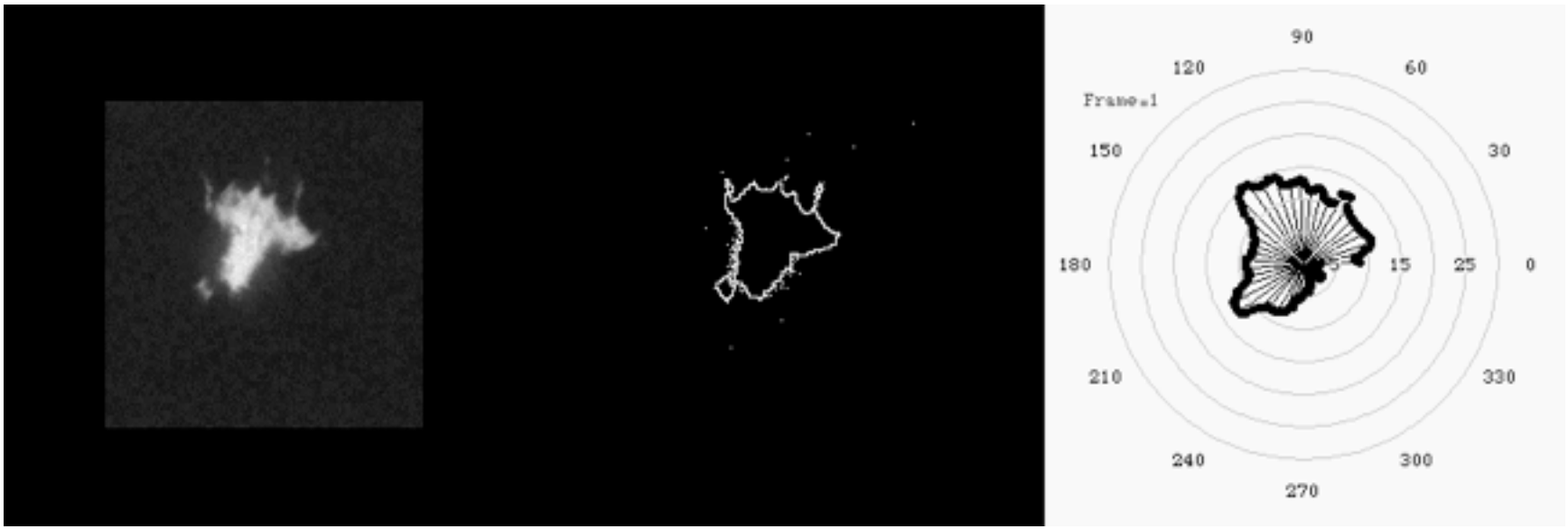


C. Sykes, J. Plastino, Institut Curie

…force generation

Simplified approach of cell functions: Cell Spreading

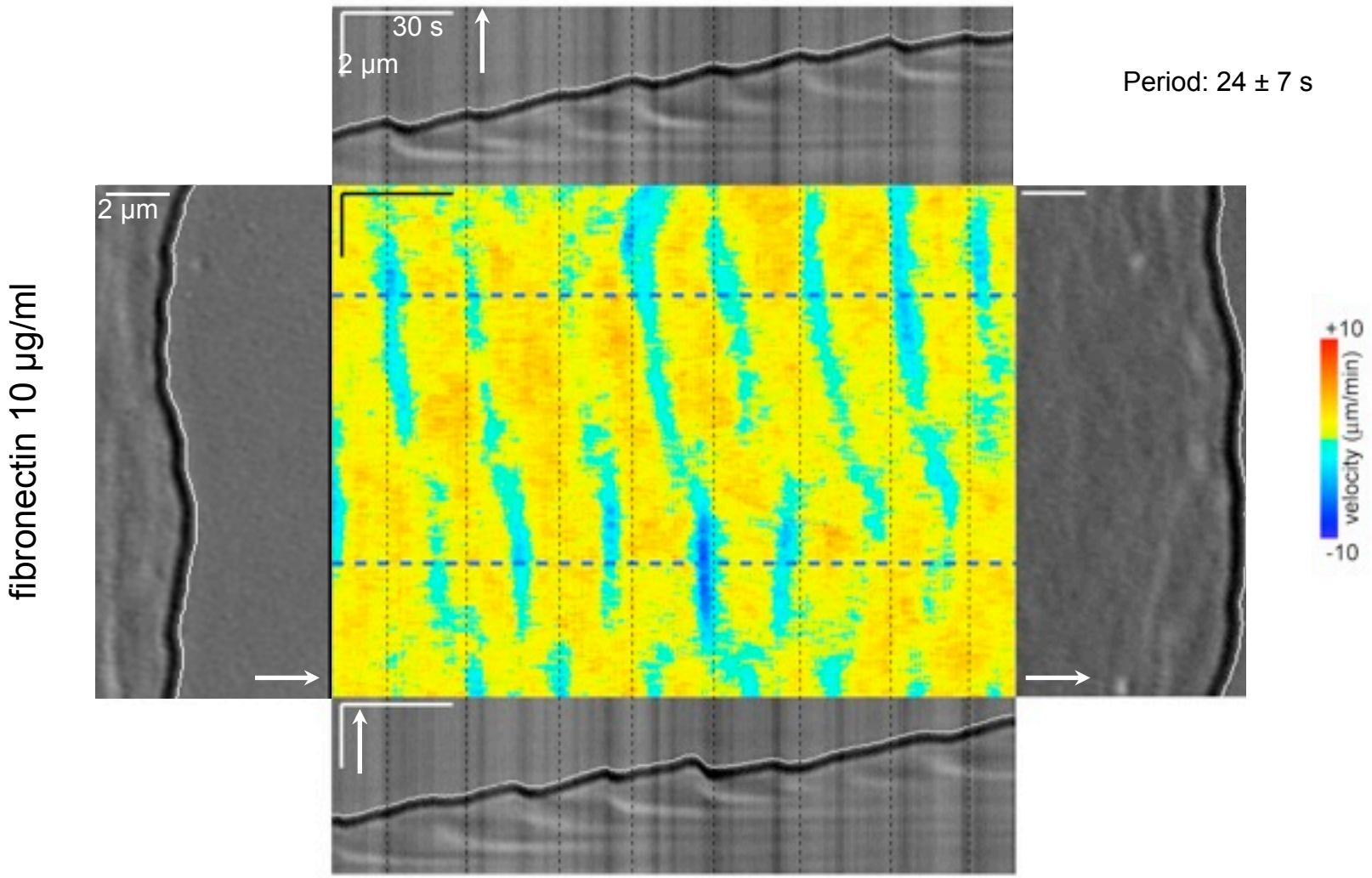
Regions of close contacts between the cell and the substrate: cell outline
Distance from the center to the edge as function of angle and time: visualization of the entire cell spreading
Derivative of the distance with respect to time: edge velocity or velocity map of the cell spreading



Dubin-Thaler et al., 2004, Biophysical J

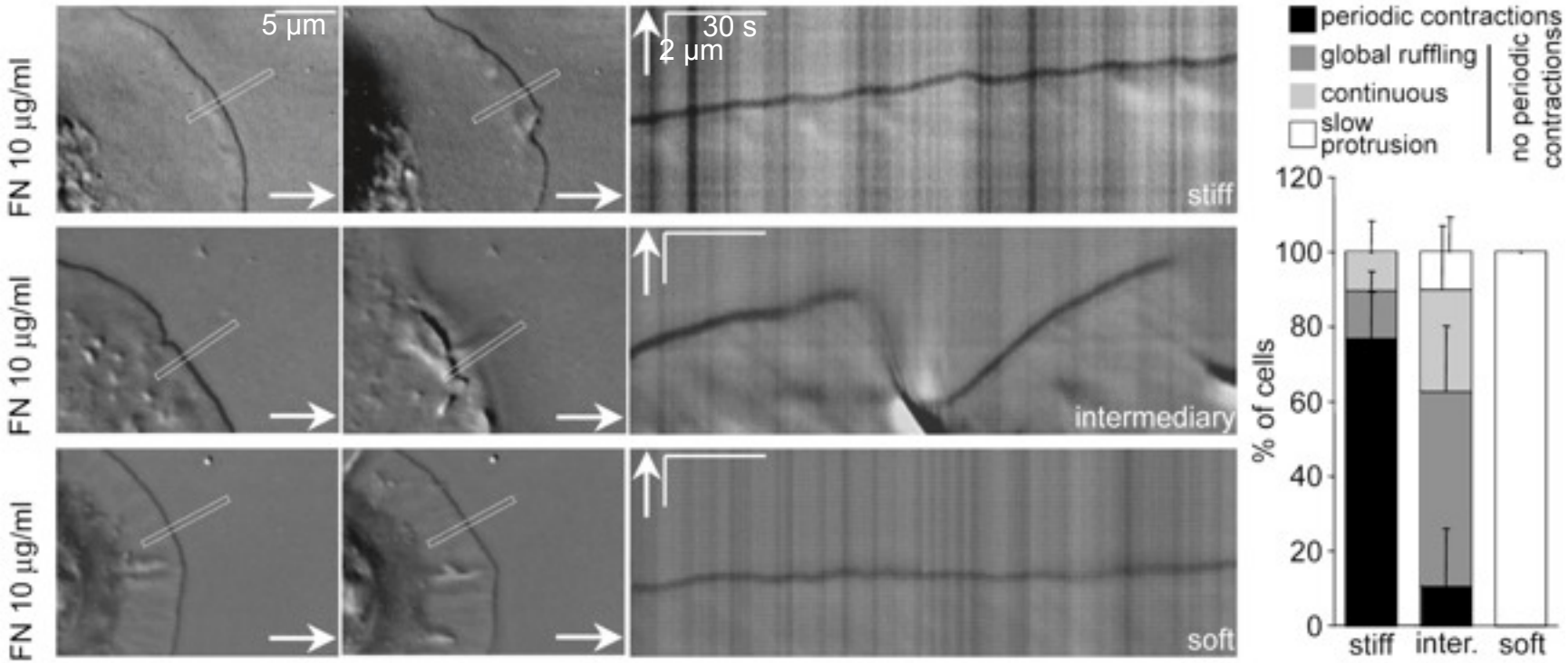
Visualisation of cell spreading (DIC)

(Giannone et al. Cell 2004)



Periodic contractions during cell spreading

Periodic contractions modulated by substrate stiffness



Quantitative biology

using **concepts and physical approaches**

reaching an equilibrium between simplification (seeking for universality!) and complexity/diversity of living matter

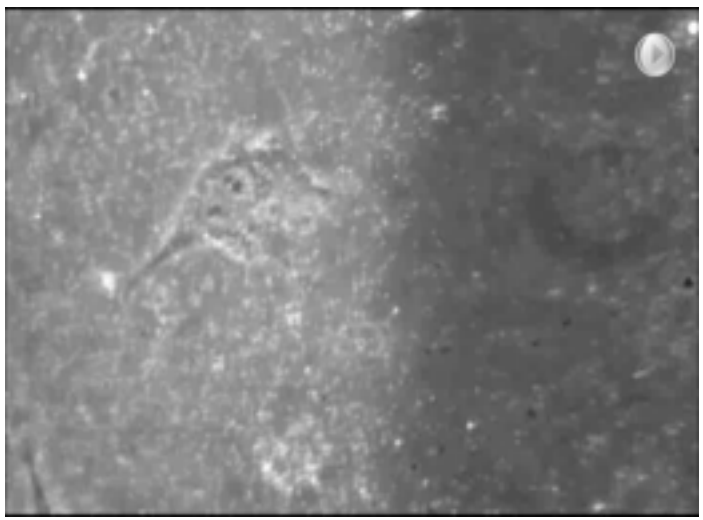
Biology by the Numbers

Rob Phillips

California Institute of Technology

Science was once called "natural philosophy" and had as its purview the scientific study of all of nature. Increasing specialization led to a splintering of natural philosophy into a number of separate disciplines and one of the outcomes of this trend was that physics emerged largely as the study of inanimate matter. This peculiar state of affairs imposed an unnatural barrier which largely prevented physicists from seeing the study of living matter as part of their core charter. Similarly, the style of analysis favored in the life sciences was often descriptive isolating much of the biological mainstream from quantitative descriptions as the rule rather than the exception. An exciting outcome of the biological revolution of the last fifty years is that the study of living matter is emerging as a true interdisciplinary science that will enrich traditional physics and biology alike. I examine some of the philosophical underpinnings of physical biology and then illuminate these ideas through several case studies that highlight the interplay between quantitative data and the models set forth to greet them. One of the interesting outcomes of an analysis from the physical biology perspective is that topics that seem very distant biologically are next door neighbors in physical biology.

Durotaxis



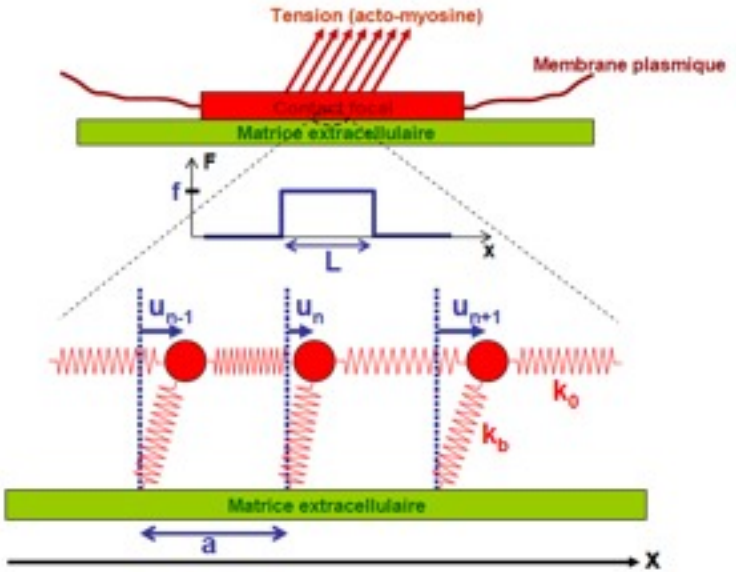
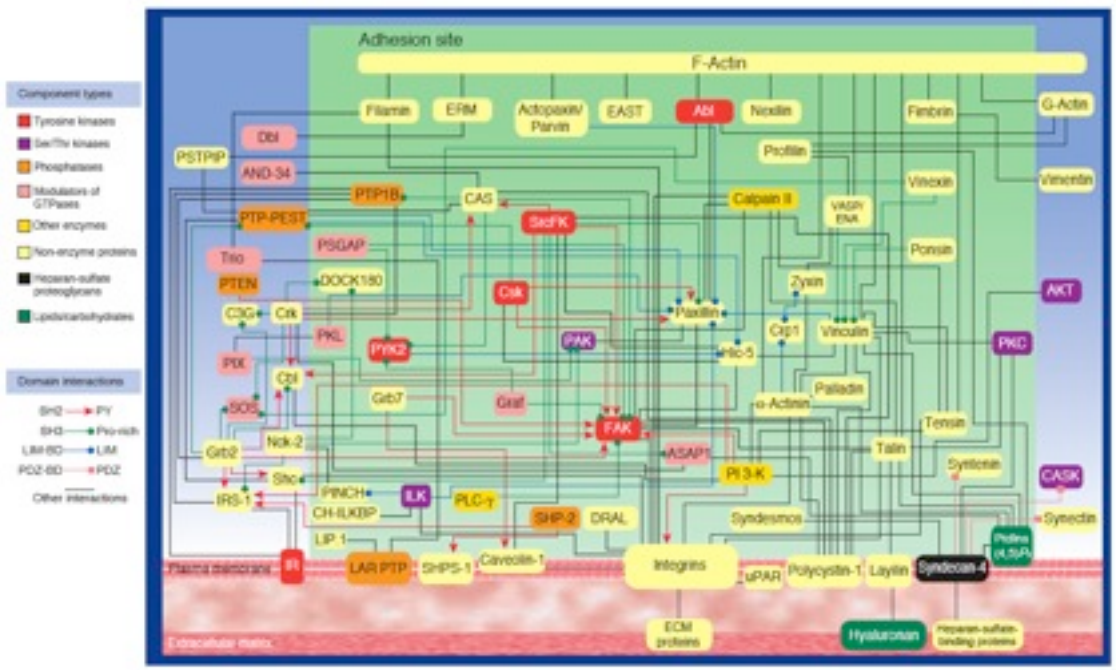


Figure 1.25: Schéma du modèle de contact focal proposé par Nicolas et al.



Modeling depends on the biological question

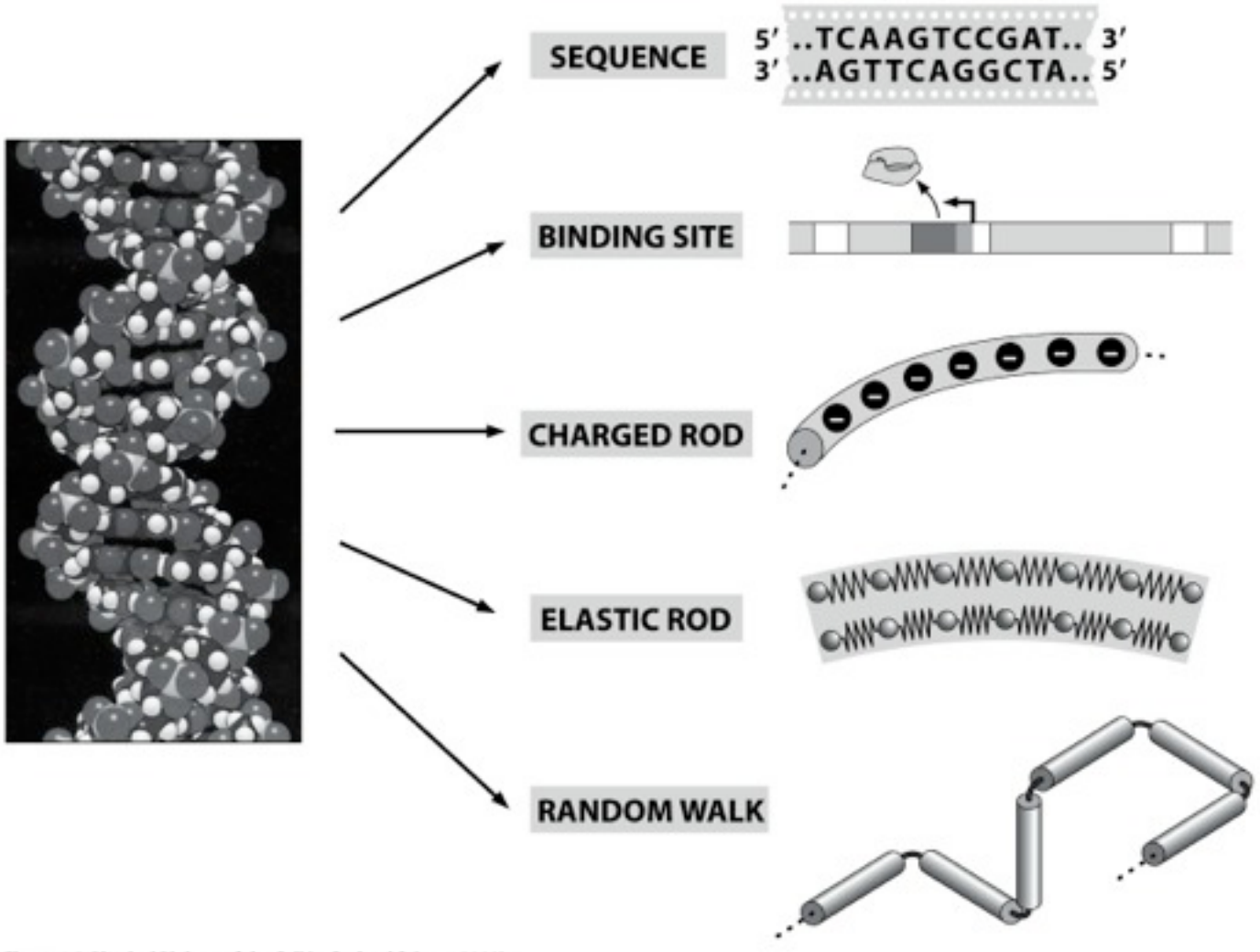
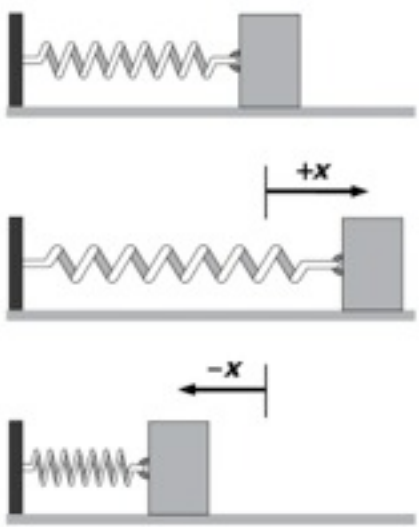
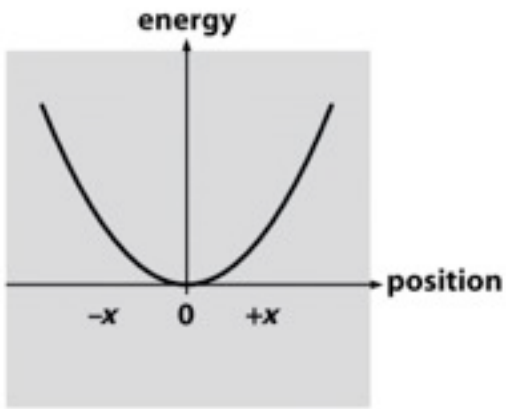


Figure 1.5 Physical Biology of the Cell (© Garland Science 2009)

Quantitative models:
« Toolbox » of the physicist

Example:
Harmonic oscillator






beam bending
(e.g., AFM cantilever)


cell membrane fluctuating


bead pulled to center
of an optical trap


molecules in an
energy landscape


DNA polymer wriggling
in solution

Figure 1.12a Physical Biology of the Cell (© Garland Science 2009)

Figure 1.12b Physical Biology of the Cell (© Garland Science 2009)

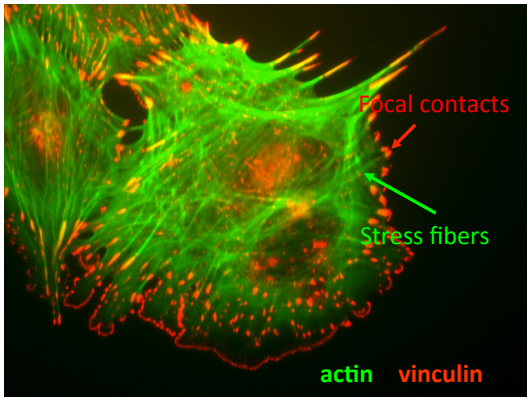
Outline

Mechanics of cell adhesion

Single cell adhesion

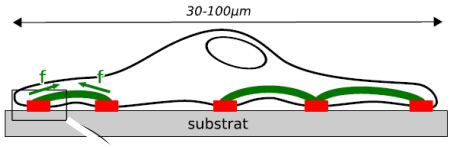
Dynamics of polymer network

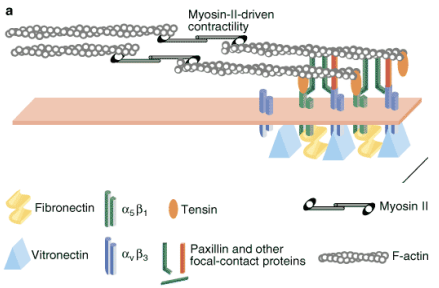
Active matter



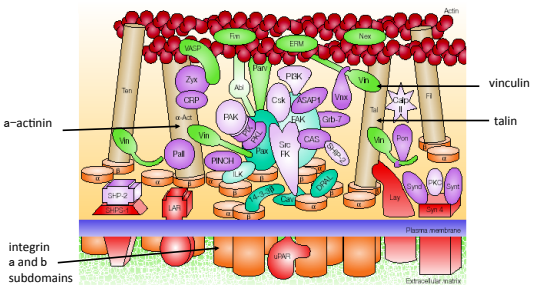
(R. Zaidel-Bar)

A. Nicolas, Images de la Physique, 2007





Complex architecture of adhesion complexes



Adapted from Geiger and Bershadsky

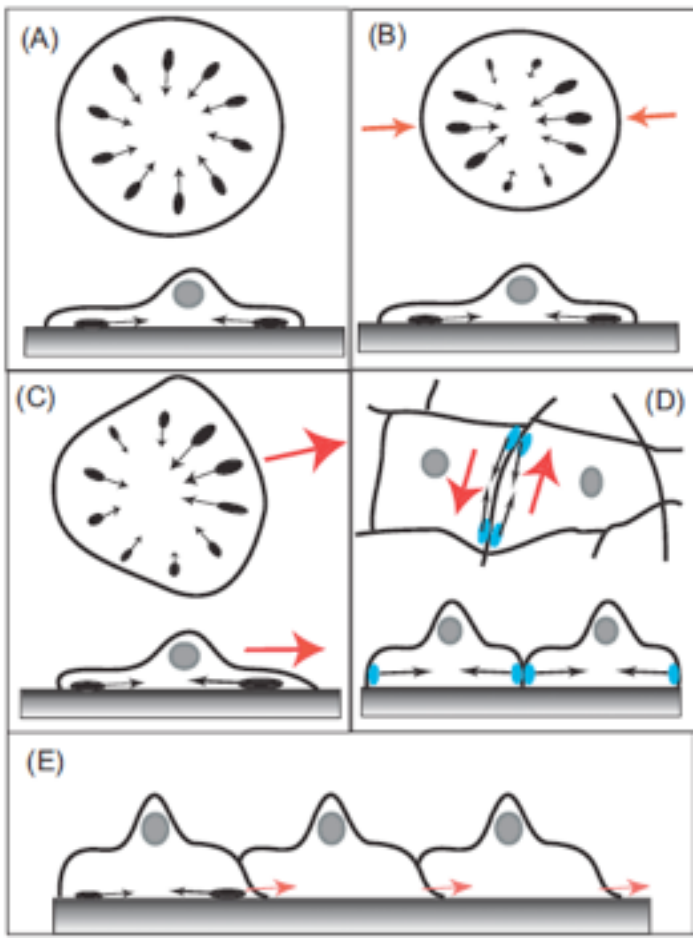
Collective cell dynamics

Interaction with neighboring cells

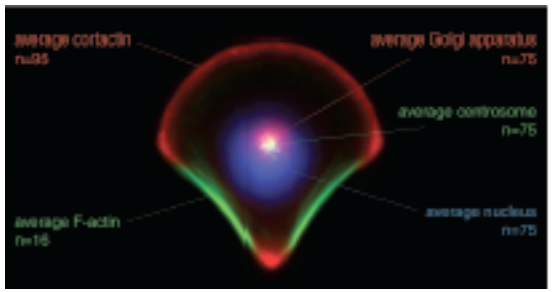
Cell colony

Multiple length scales and time scales?

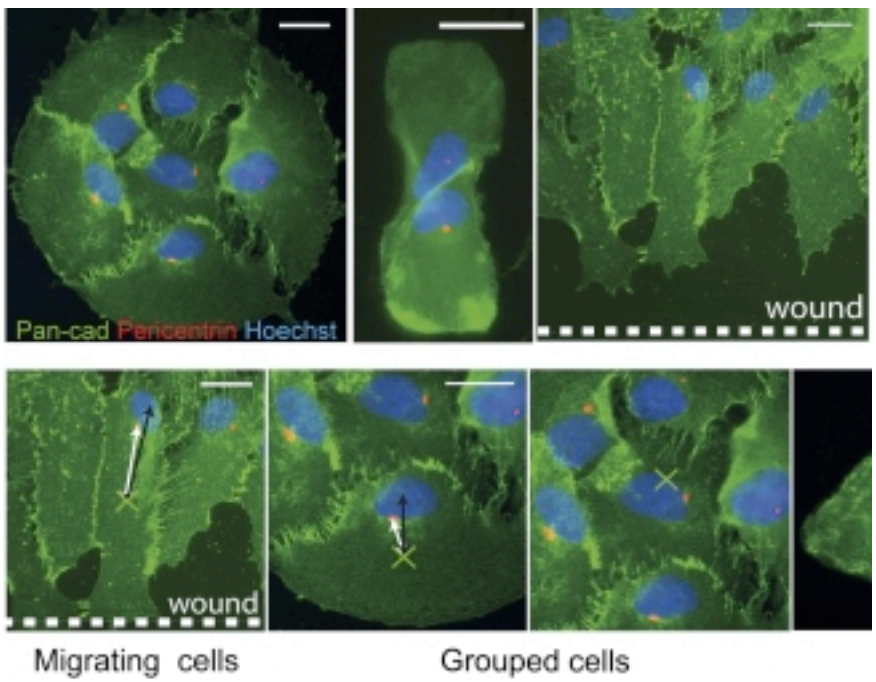
Cell organization and traction forces



Nir Gov HFSP J. 2009



Thery et al. PNAS 2005



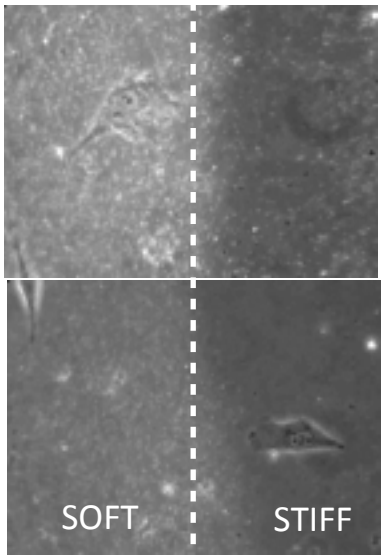
Dupin et al. JCB 2009

Mechanosensing: Active matter

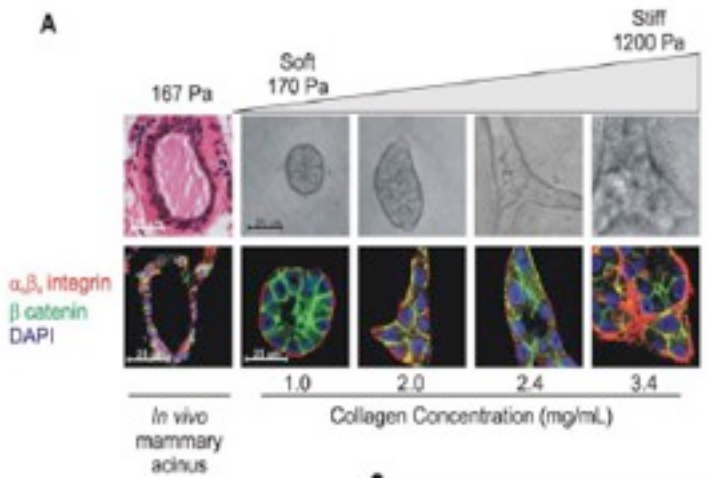
Cells can sense mechanical cues from their environment

- Different mechanical cues – Topography, Geometry, Rigidity
- Rigidity + External forces

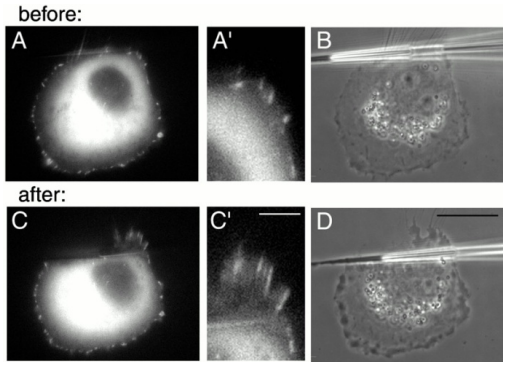
Cell migration
(Lo et al 2000)



Tumor progression
(Pascek et al 2006)

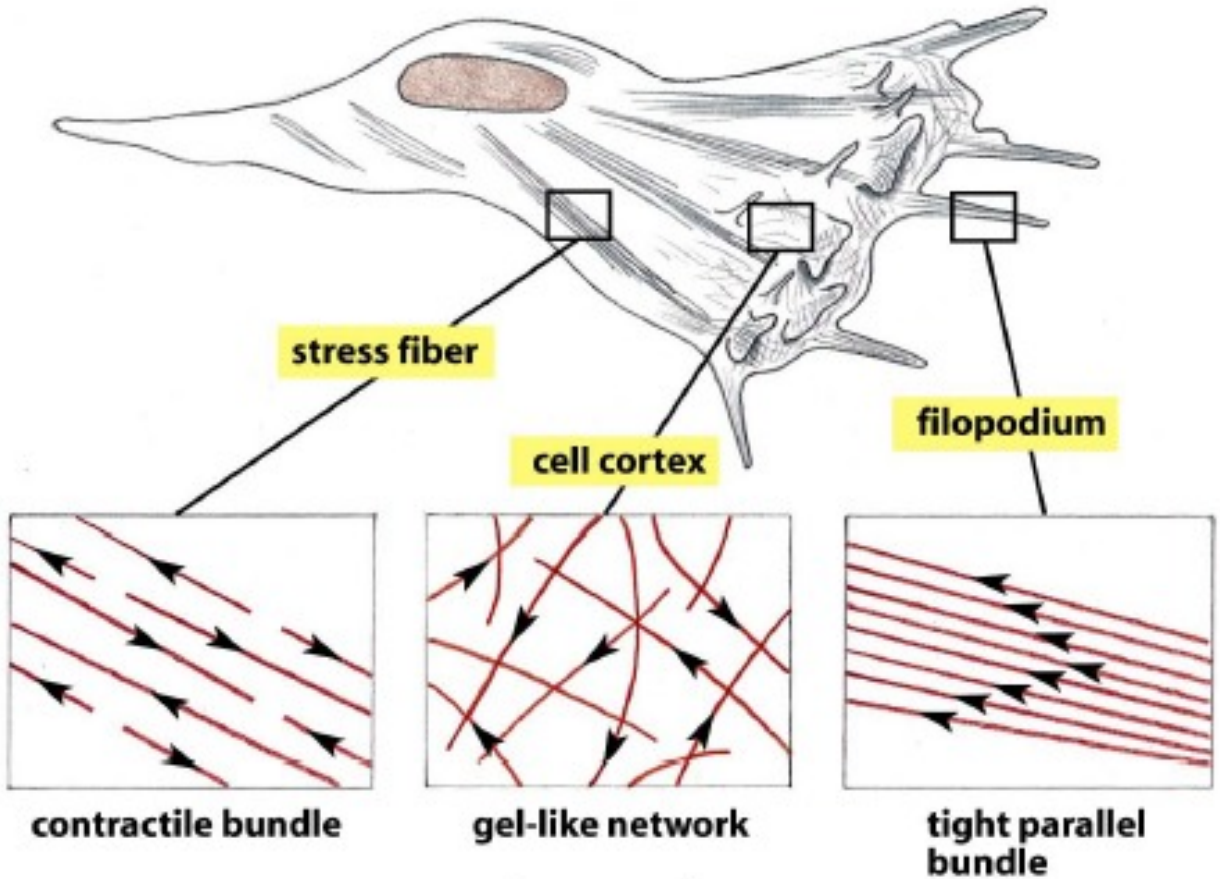


Cell adhesion
(Riveline et al 2001)



Potential applications in medicine, tissue engineering and re-generation, cancer therapy

Actin organization inside the cell



Cytoskeleton filaments are polymers, made of small protein subunits

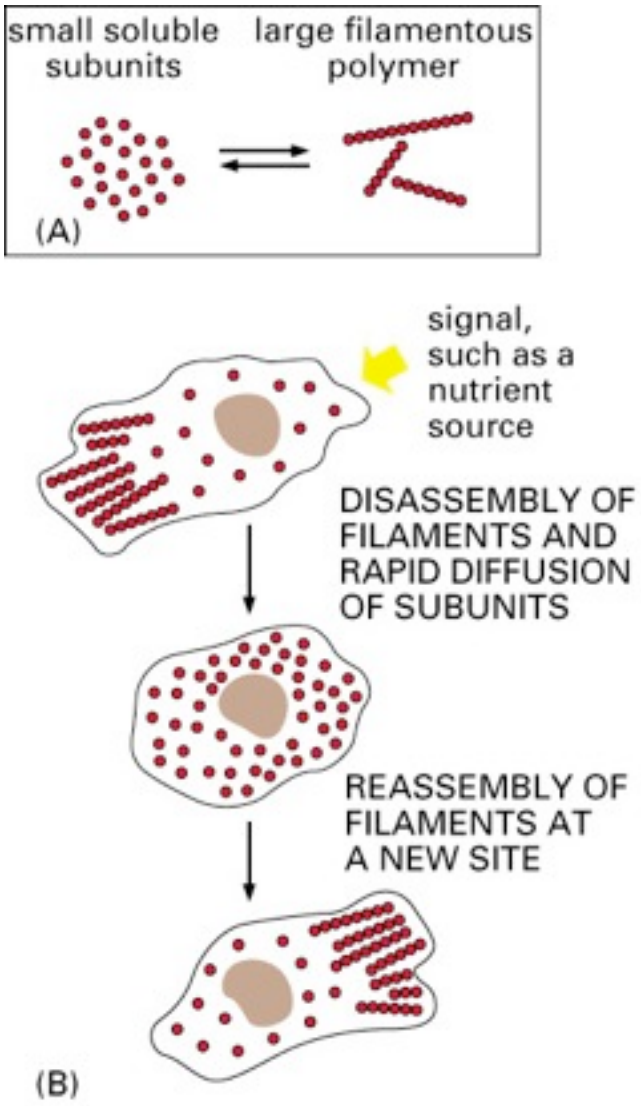


Figure 16–2. Molecular Biology of the Cell, 4th Edition.

1. The subunits are small and can diffuse in the cytosol whereas polymers cannot.
   Cells can undergo rapid structural reorganization

2. The polymers are held by weak non-covalent linkages
   Assembly and disassembly can occur rapidly

3. Accessory proteins regulate the spatial distribution and dynamics of filaments and monomers
   Bring cytoskeletal structures under the control of extra- and intracellular signals

Passive polymerisation (w/o ATP):
equilibrium polymer

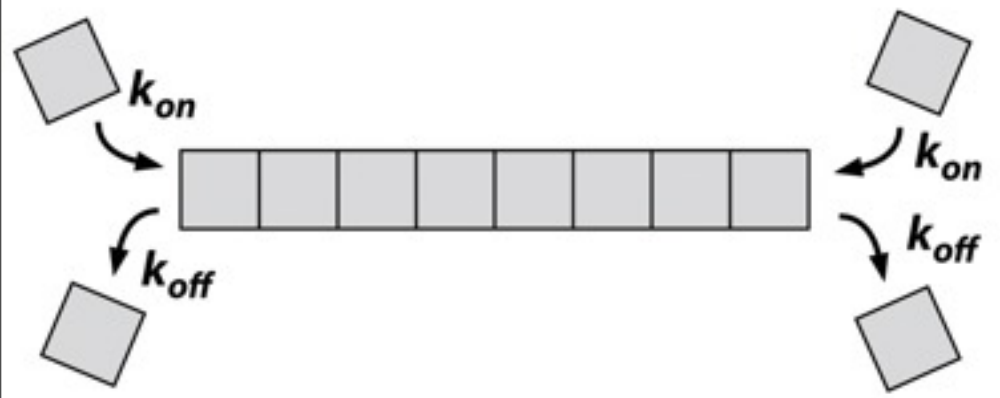


Figure 15.21a Physical Biology of the Cell (© Garland Science 2009)

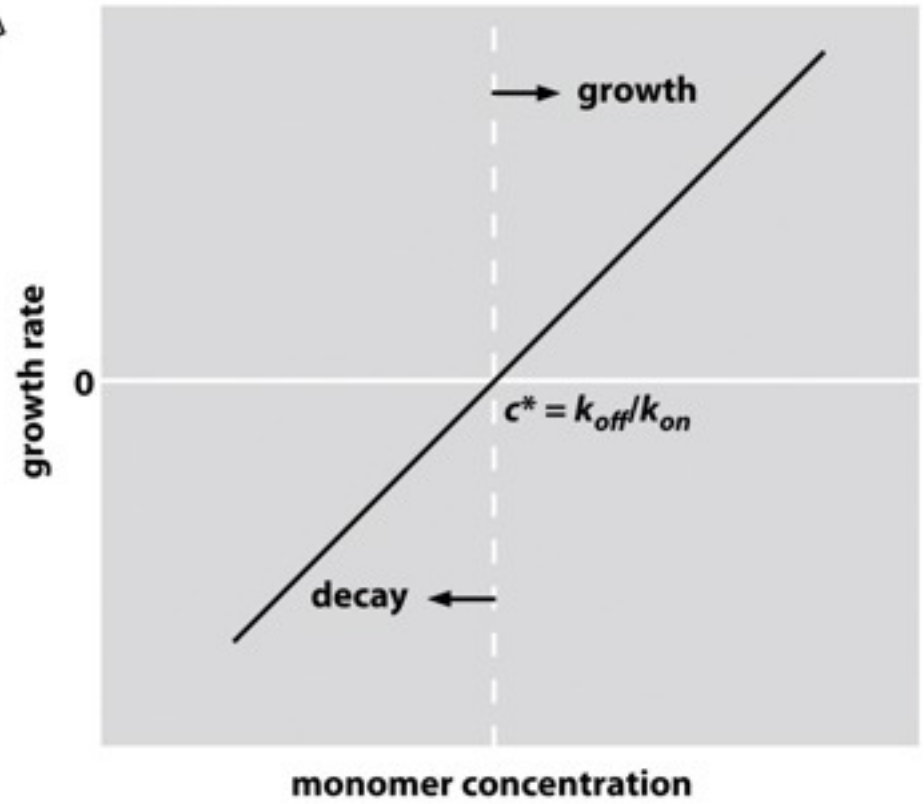
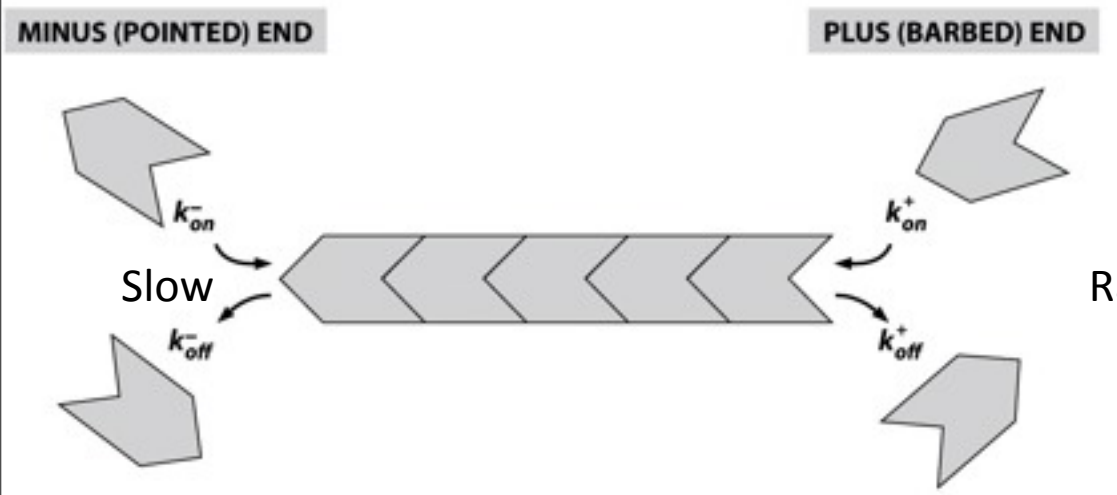
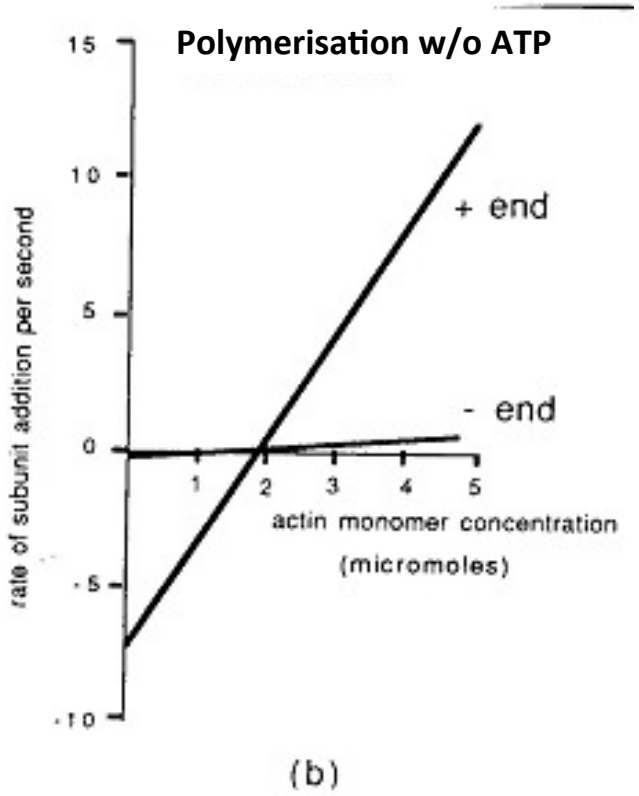


Figure 15.29 Physical Biology of the Cell (© Garland Science 2009)

Passive polymerisation (w/o ATP):
But difference between both ends of the filament





Dissymetric case

k_{on} and k_{off} ≠ at both ends

" + " = barbed end → rapid

" − " = pointed end → slow

Same chemical reaction (same result if one monomer is added from the left or from the right)

$$\Delta G^+ = \Delta G^-$$

$$\frac{k_{on}^+}{k_{off}^+} = \frac{k_{on}^-}{k_{off}^-}$$

$\begin{cases} n^+ = \text{added at } \oplus \text{ end} \\ n^- = \text{added at } \ominus \text{ end} \end{cases}$

$$\begin{cases} \frac{dn^+}{dt} = k_{on}^+ c_1 - k_{off}^+ \\ \frac{dn^-}{dt} = k_{on}^- c_1 - k_{off}^- \end{cases} \Rightarrow \text{same } \underline{\underline{c^*}} \quad \left(\frac{dn^i}{dt} = 0 \right)$$

Active polymerisation (with ATP)

Different on- and off-rates for ATP- or ADP- monomers

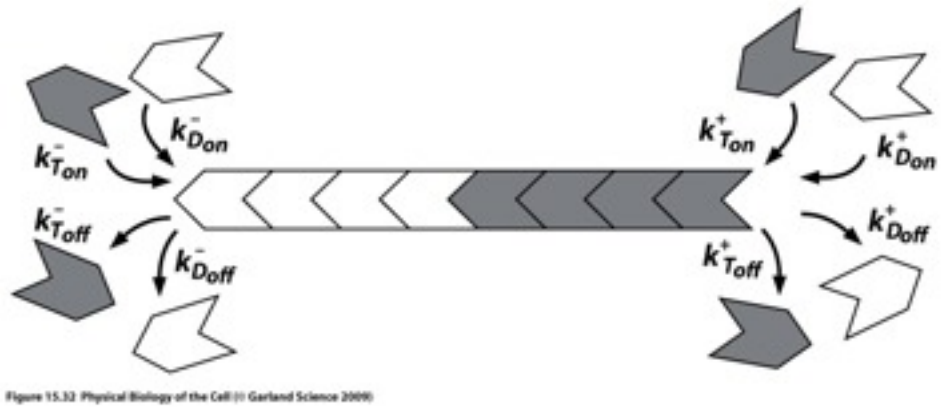


Figure 15.32 Physical Biology of the Cell (© Garland Science 2009)

Treadmilling

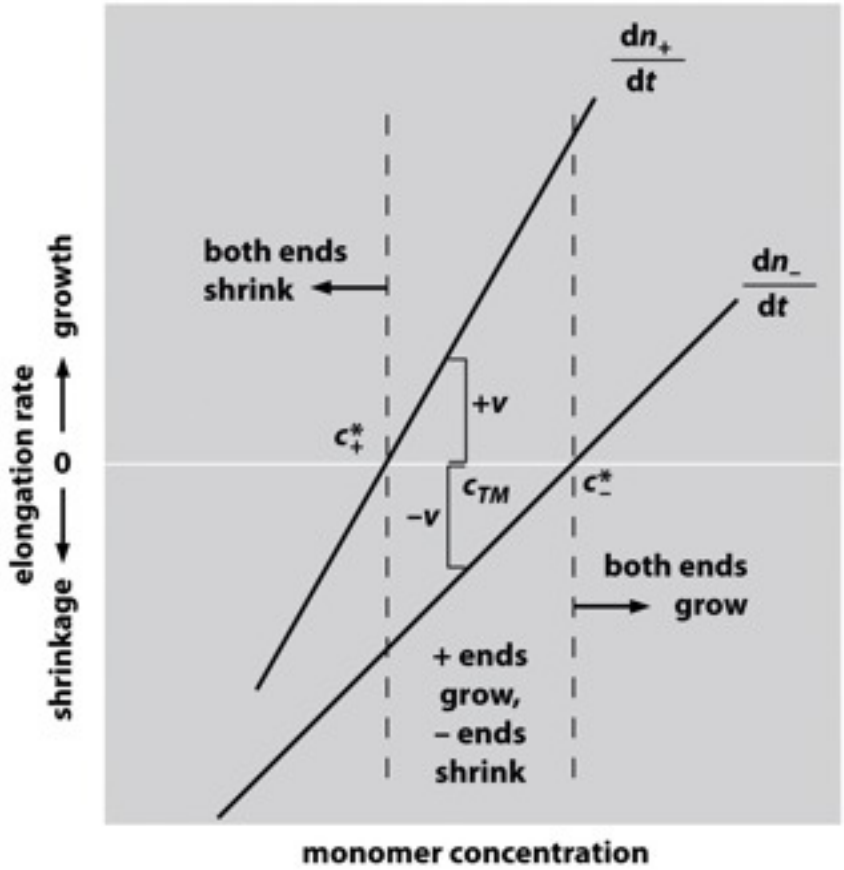
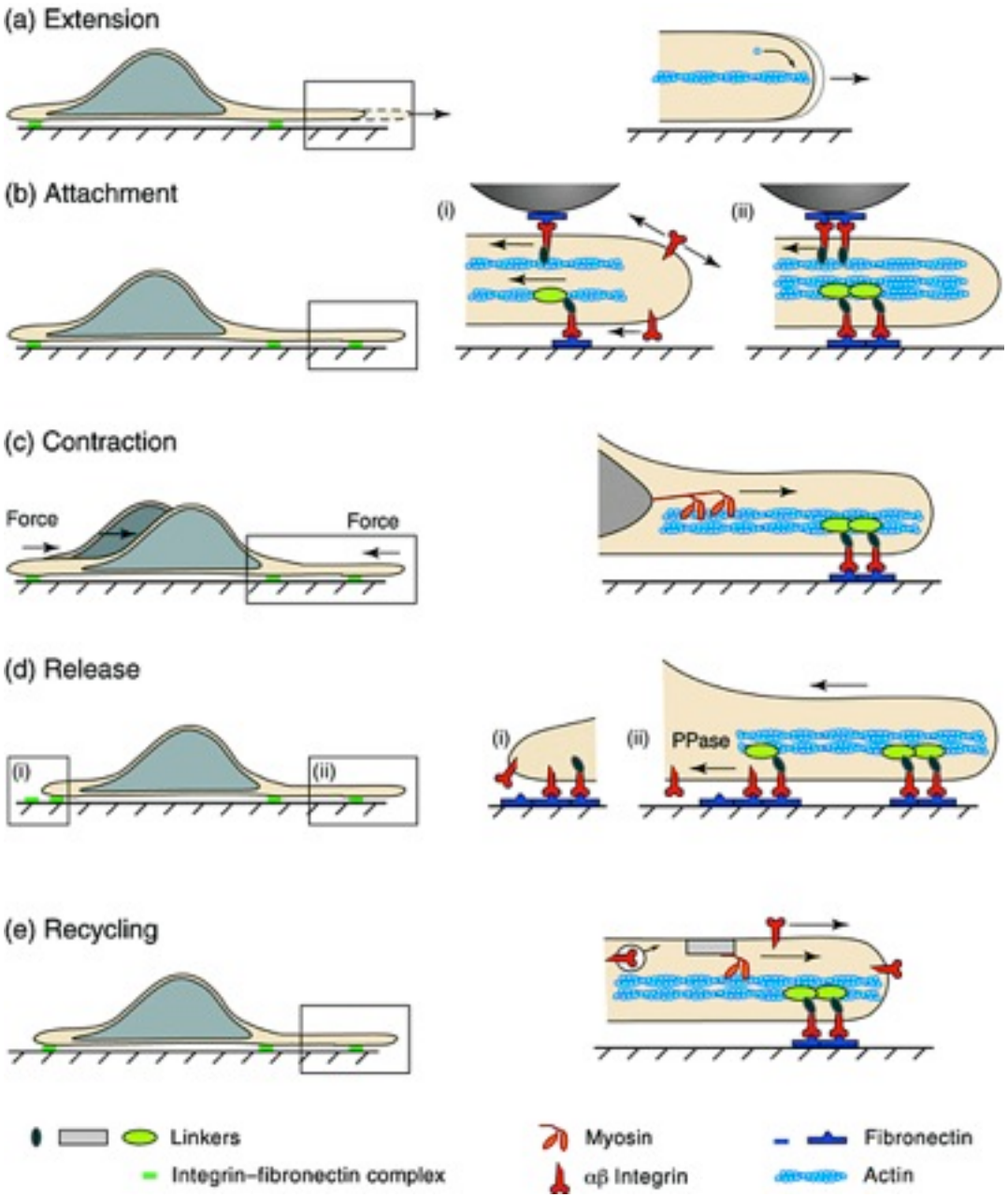


Figure 15.33a Physical Biology of the Cell (© Garland Science 2009)

Single cell migration: a cyclic process of force generation



lamellipodial extension:
forward force exerted by actin polymerization on the membrane

extracellular matrix probing:
rearward force exerted by actin flow on integrins

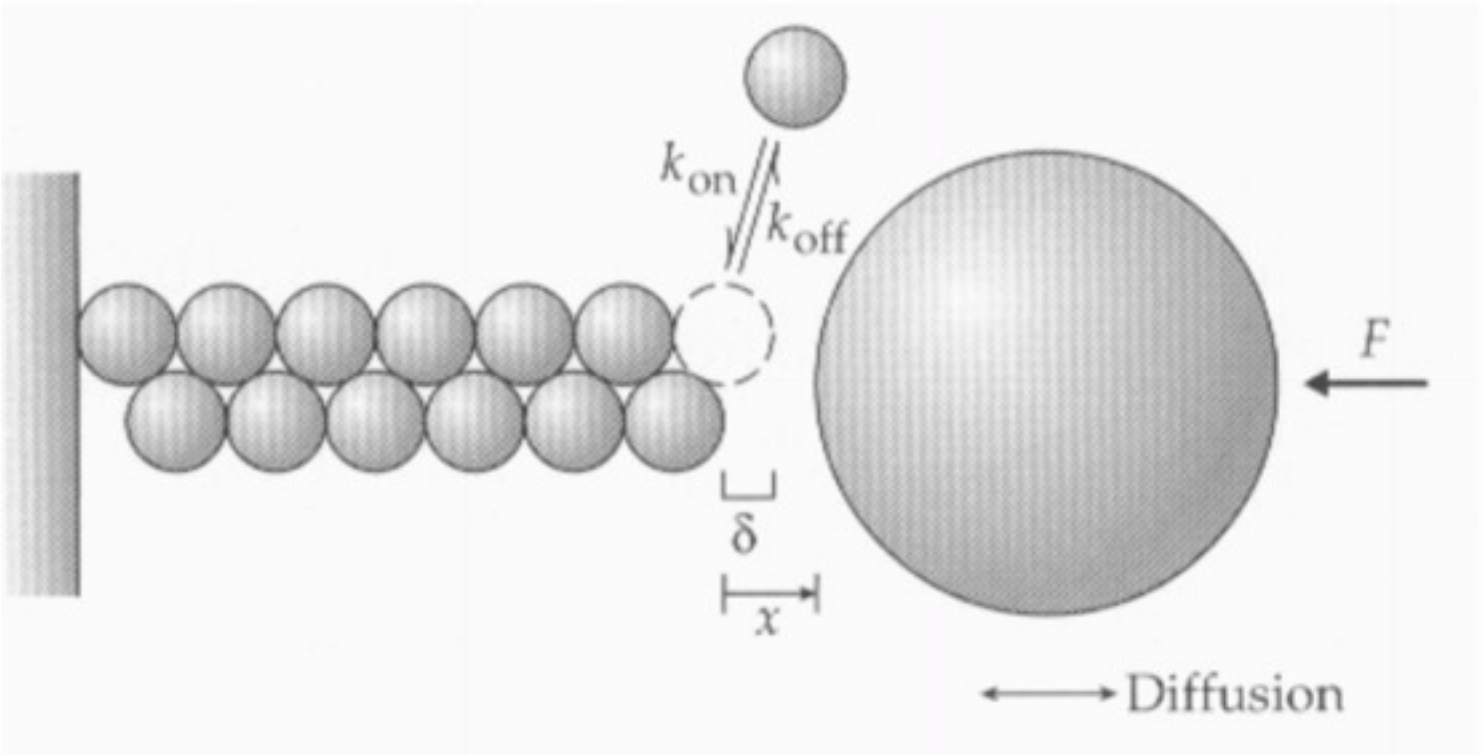
How the cell transduces mechanical signals into a biochemical signal?

Sheetz M.P., Felsenfeld D.P., Galbraith C.G., 1998, Trends Cell Biol, 8:51-4

Force generated by polymerisation

Force exerted by filament growth against the cell membrane?
Inversely, how an external force can affect actin polymerisation?

(See Oster, Mogilner)



Force generated by polymerisation

* Growth velocity of the filament

$$v = \delta\,(k_{on}\,c - k_{off})$$

c: Monomer concentration

δ: Added length by adding a new monomer

* Thermodynamics

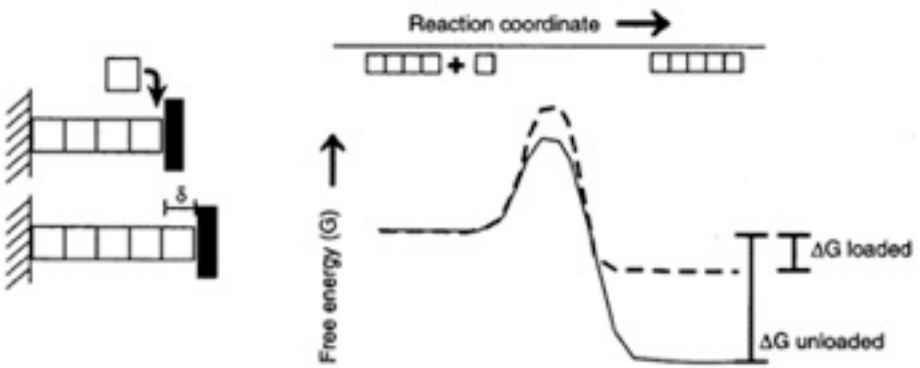
$$\Delta G\,(F=0) \rightarrow \Delta G(F) = \Delta G(0) - F\delta$$

$$k_d(F) = k_d(0)\; e^{+F\delta/kT}$$

$$\frac{k_{off}(F)}{k_{on}(F)} = \frac{k_{off}(0)}{k_{on}(0)}\; e^{+F\delta/kT}$$

⚠ Sign F F>0, compressive force

depolymerisation ↗ $k_d(F)$ ↗



The Polymerization Motor

Julie A. Theriot *Traffic 2000* **1**: 19–28

$$v(F) = \delta \left\{ k_{on} c \exp\left(-q \frac{F\delta}{kT}\right) - k_{off} \exp\left((1-q)\frac{F\delta}{kT}\right) \right\}$$

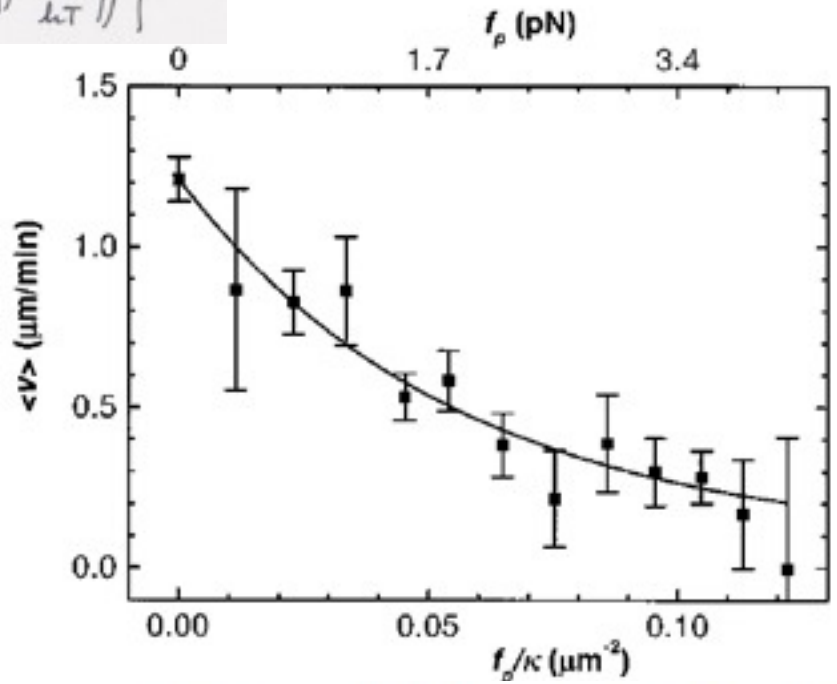
$0 \leq q \leq 1$

$q = 0$ $\quad k_{on}(F) = k_{on}(0)$

only k_{off} is affected by F

$q = 1$ $\quad k_{off}(F) = k_{on}(0)$

only k_{on} is affected by F



Measurement of the Force-Velocity Relation for Growing Microtubules

Marileen Dogterom* and Bernard Yurke

SCIENCE • VOL. 278 • 31 OCTOBER 1997

Experimentally: $k_{off}(F) \simeq k_{off}(0)$

External force reduces k_{on} (mostly)

$$v(F) = \delta \left[k_{on}(0)\, e^{-F\delta/kT} - k_{off}(0) \right]$$

stall Force for $v = 0 \Rightarrow$ $\quad F = \frac{k_B T}{\delta} \ln\left(\frac{k_{on}(0)\, c}{k_{off}(0)}\right)$

$\Rightarrow$ $\quad F_{stall} = \frac{k_B T}{\delta} \ln\left(\frac{c}{K_d(0)}\right)$

Orders of magnitude

Actin inside the cell, m= 20 µM

$K_d(2) = m^* = 0.2\,\mu M$

$\delta = 5.4\,nm / 2$ protofilaments $= 2.7\,nm$

$F_{max} = \frac{4\,pN.nm}{2.7\,nm} \ln 100 \simeq 7\,pN$

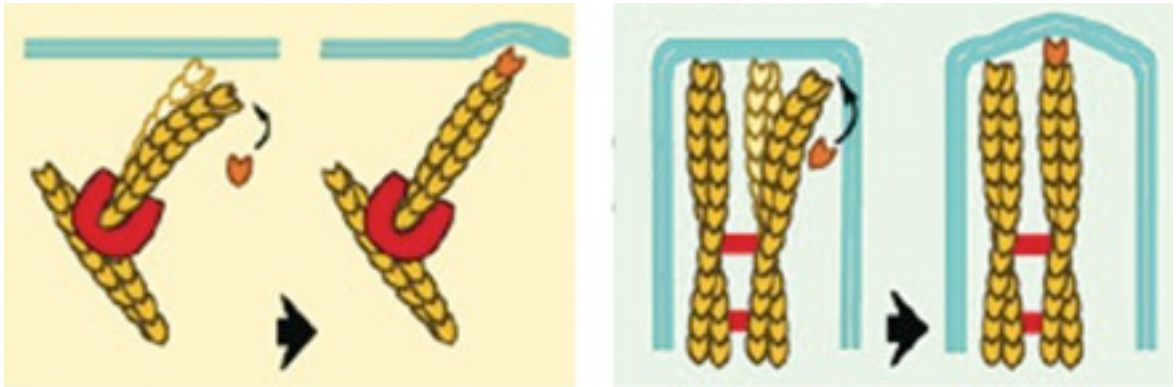
Microtubule : $m \simeq 100\, m^*$

$\delta = 8/13 \simeq 0.6\,nm$

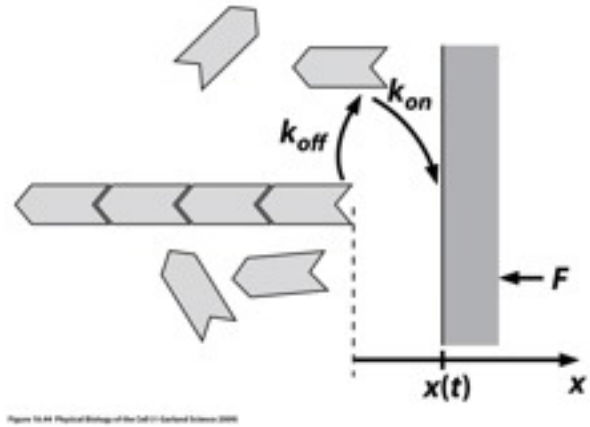
$F_{max} = \frac{4\,pN.nm}{0.6\,nm} \ln 100 \simeq 30\,pN$.

Force generated by polymerisation

Brownian ratchet model:



[G. Borisy & T. Svitkina,*Curr. Opin. Cell Biol.*, 12:104-112 (2000)]



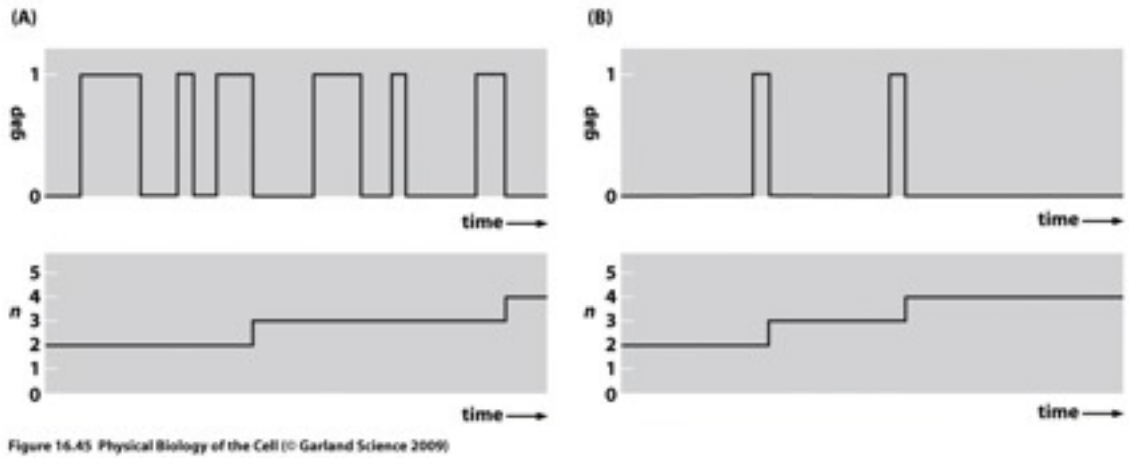
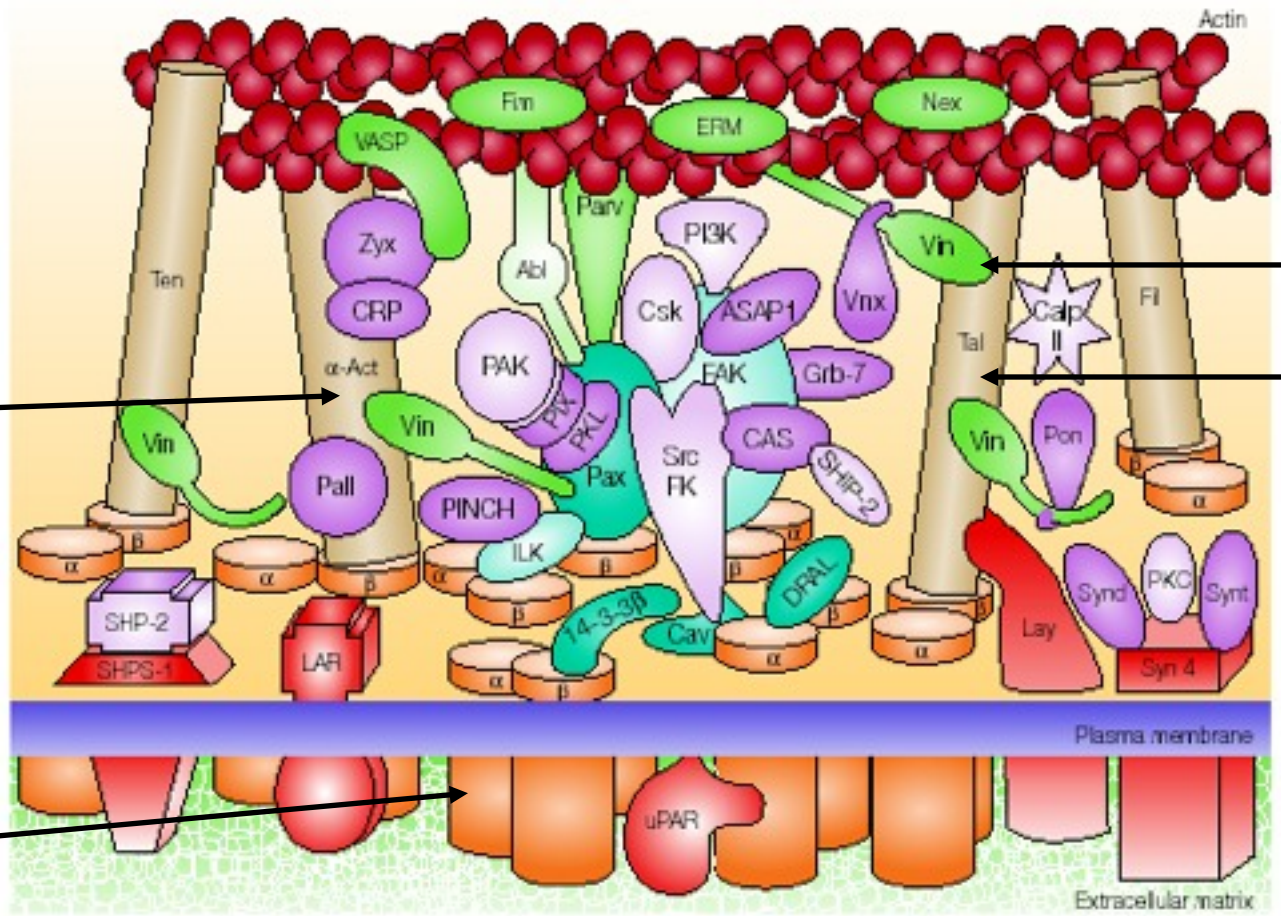


Figure 16.45 Physical Biology of the Cell (© Garland Science 2009)

Mechanism limited either by 1) probability to add a new monomer 2) or gap opening

The complex architecture of focal adhesion



vinculin

talin

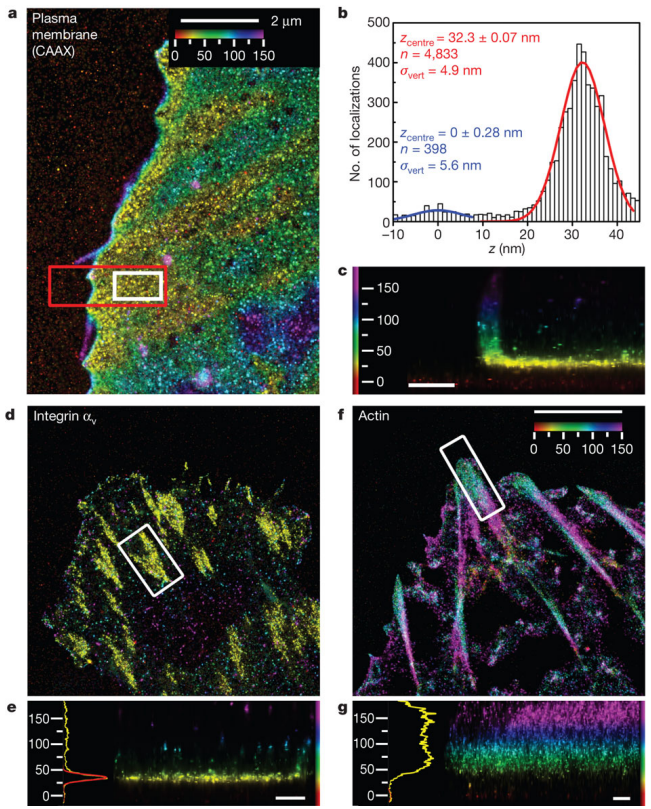
α–actinin

integrin α and β subdomains

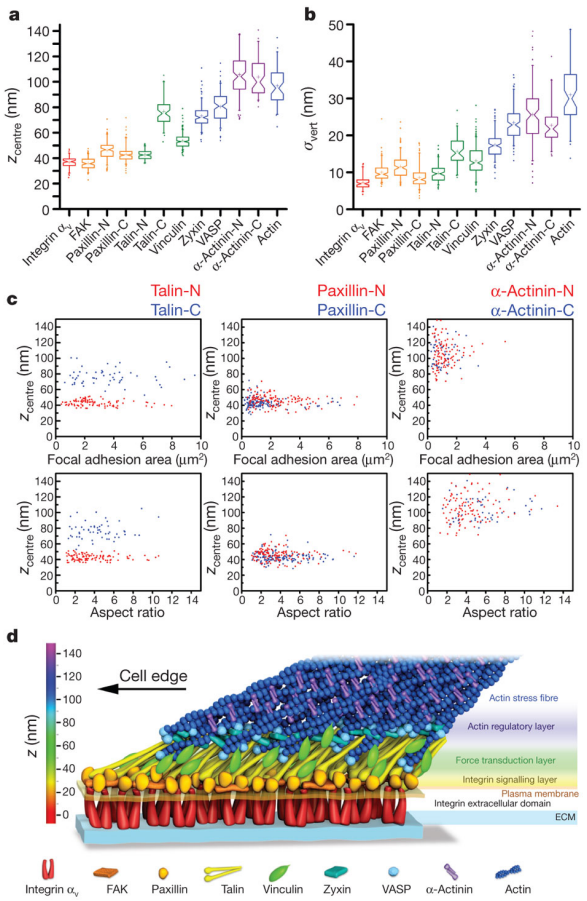
Adapted from Geiger and Bershadsky

Nanoscale architecture of FAs

iPALM imaging:
plasma membrane marker, integrin and actin.

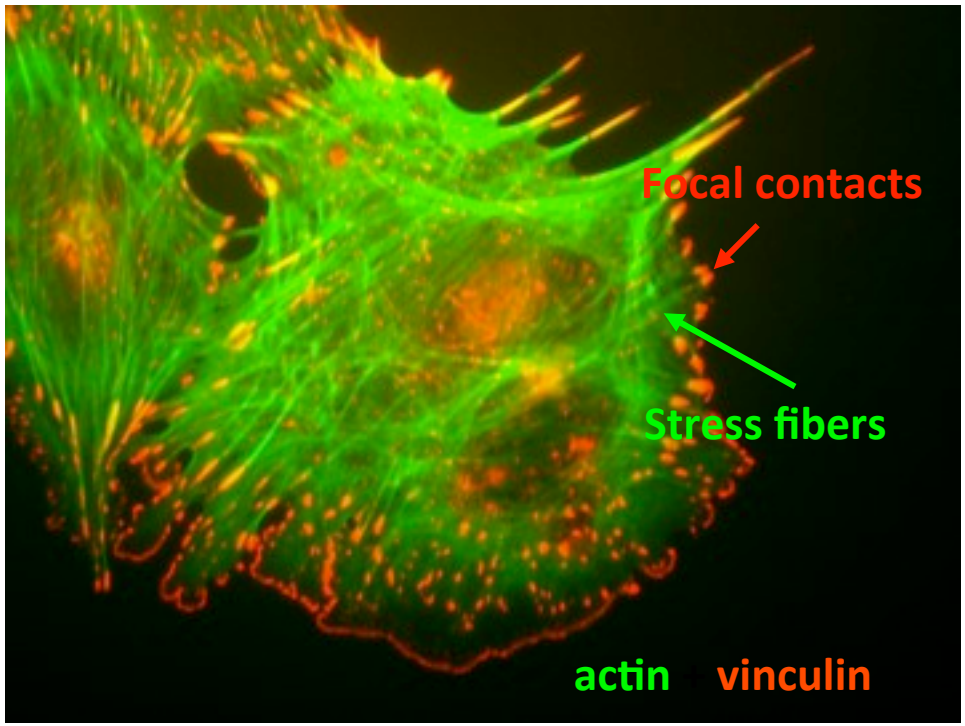


Distribution & position of molecules



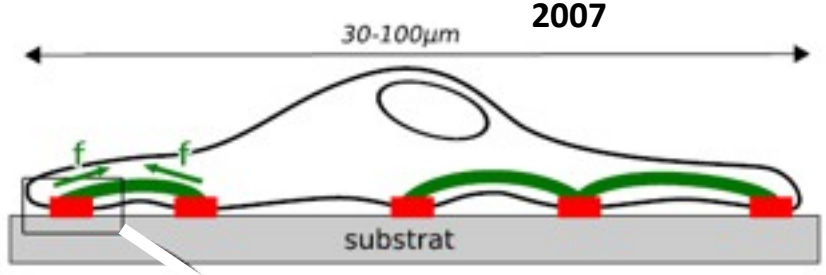
Kanchanawong *et al. Nature* **468**, 580-584 (2010)

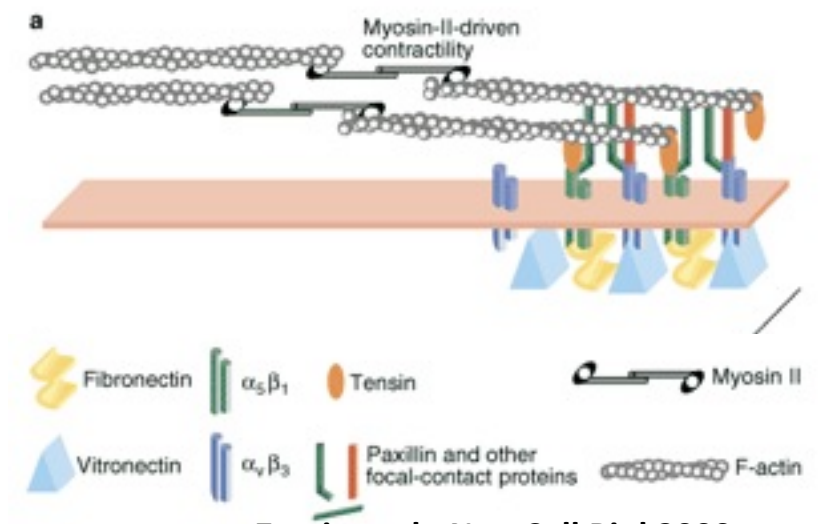
Adhesion, contractility and transmission of forces



(R. Zaidel-Bar)

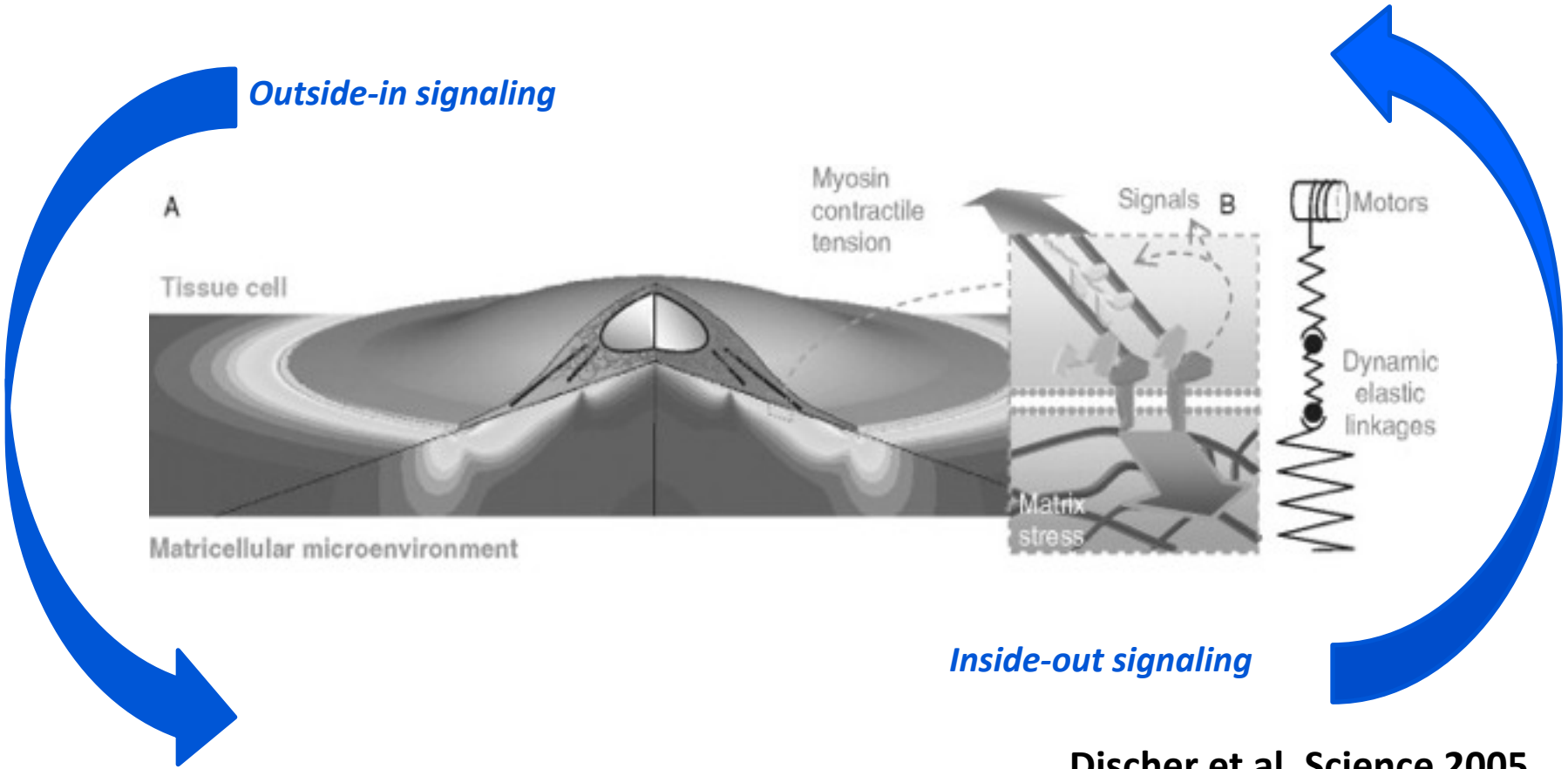
A. Nicolas, Images de la Physique, 2007





Zamir et al., Nat. Cell Biol 2000

How the cell transduces external mechanical signals of the ECM into a biochemical one?



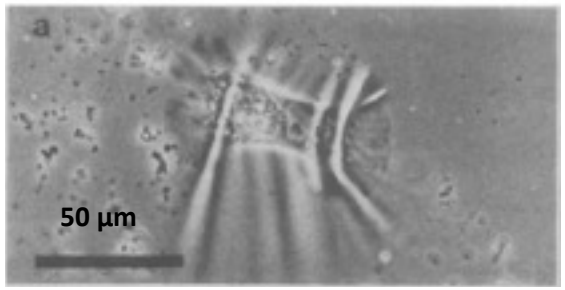
Discher et al. Science 2005

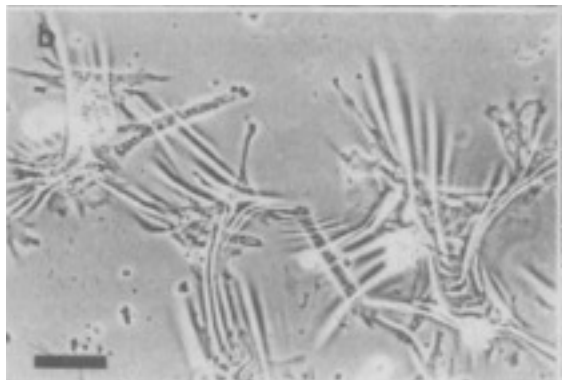
Mechano-sensing process ? Protein stretching, ion channels, actin cytoskeleton…

Force mapping on the substrate : different techniques

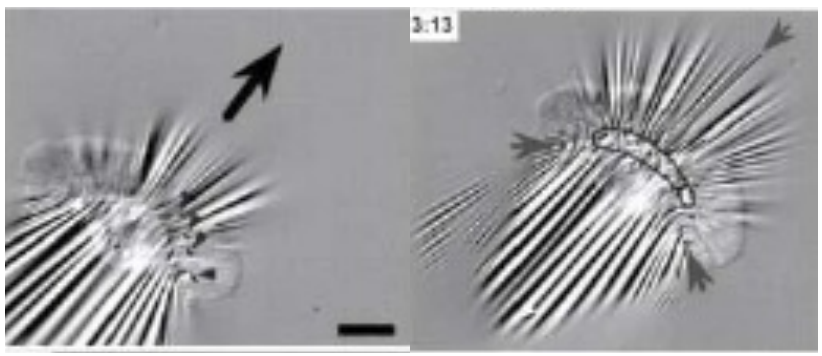
Wrinkles on a silicon layer: Qualitative data

Harris *et al., Science*, 1980





Cell on elastic deformable substrate (silicon)



Migrating cell (keratocyte)

Burton et al. Mol. Biol. Cell 10 p. 3745 (1999)

force ~ nN ; size ≈≈ µm Other methods?

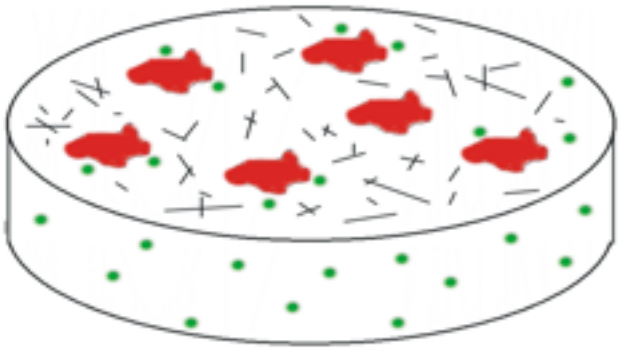
Adaptation of Polyacrylamide Materials as Substrates for Cell Mechanical Studies

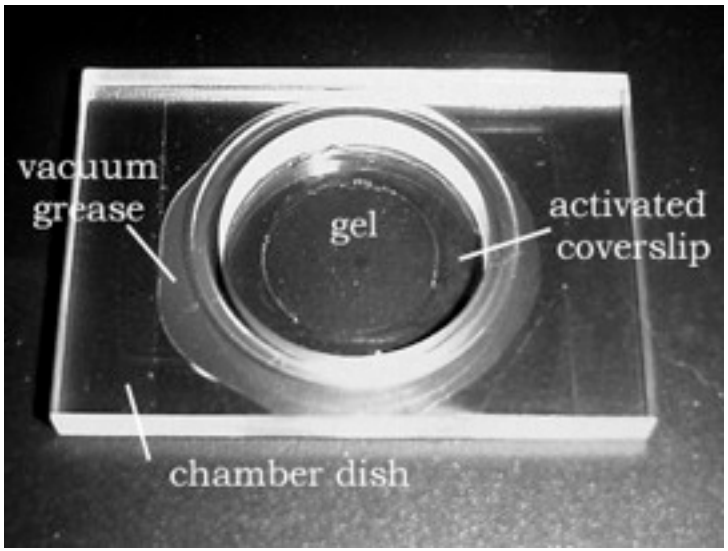
-optically clear

-10^2 – 10^3 measurements / cell

-spatial precision of bead displacement 50-100 nm

-temporal resolution ≈0.5 min



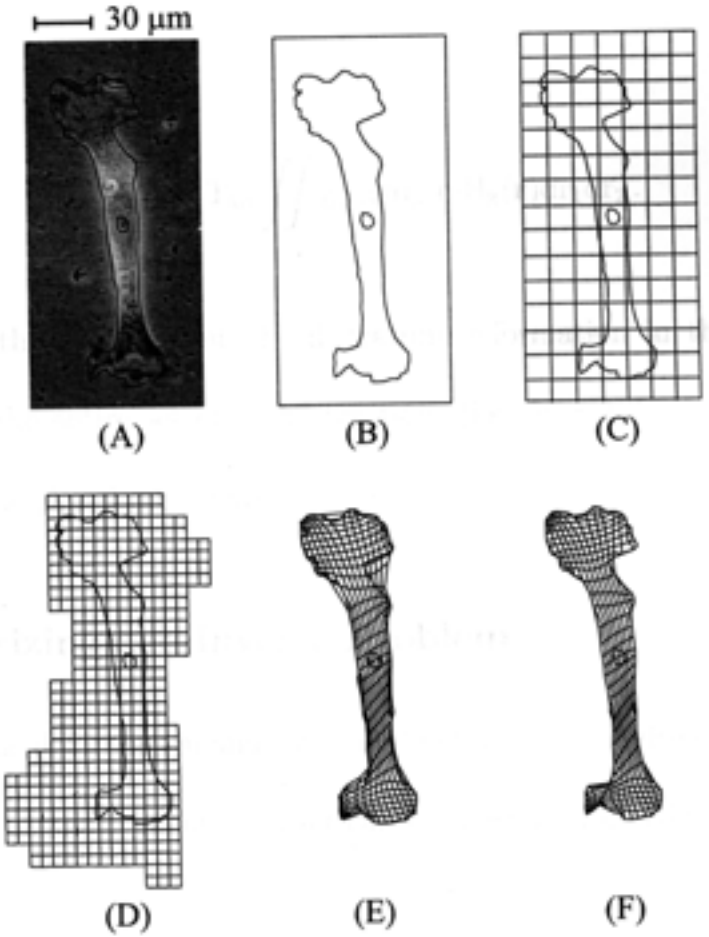


Wang and Pelham (1998) Methods Enzymol. 298:489
Beningo et al. (2002) Methods Cell Biol. 69:325

Forces Generated by a Migrating 3T3 Fibroblast Are Detected as Displacements of Particles Embedded in the Flexible Substrate

Munevar et al. (2001) Biophys. J. 80:1744

Constructing a Quadrilateral Mesh and Solving the Boussinesq Equations



D = AT

$$d_{p\alpha} = \iint g_{\beta\alpha}(\mathbf{m}_p - \mathbf{r}) T_\beta(\mathbf{r})\, dr_1\, dr_2 .$$

$$g_{11} = \frac{1+\upsilon}{2\pi E}\left(\frac{(2(1-\upsilon)r - x_3)}{r(r-x_3)} + \frac{(2r(\upsilon r - x_3) + x_3^2)x_1^2}{r^3(r-x_3)^2}\right)$$

$$g_{21} = \frac{1+\upsilon}{2\pi E}\left(\frac{(2r(\upsilon r - x_3) + x_3^2)x_1 x_2}{r^3(r-x_3)^2}\right)$$

$$g_{31} = \frac{1+\upsilon}{2\pi E}\left(\frac{x_1 x_3}{r^3} + \frac{(1-2\upsilon)x_1}{r(r-x_3)}\right)$$

$$g_{12} = \frac{1+\upsilon}{2\pi E}\left(\frac{(2r(\upsilon r - x_3) + x_3^2)x_1 x_2}{r^3(r-x_3)^2}\right)$$

$$g_{22} = \frac{1+\upsilon}{2\pi E}\left(\frac{(2(1-\upsilon)r - x_3)}{r(r-x_3)} + \frac{(2r(\upsilon r - x_3) + x_3^2)x_2^2}{r^3(r-x_3)^2}\right)$$

$$g_{32} = \frac{1+\upsilon}{2\pi E}\left(\frac{x_2 x_3}{r^3} + \frac{(1-2\upsilon)x_2}{r(r-x_3)}\right)$$

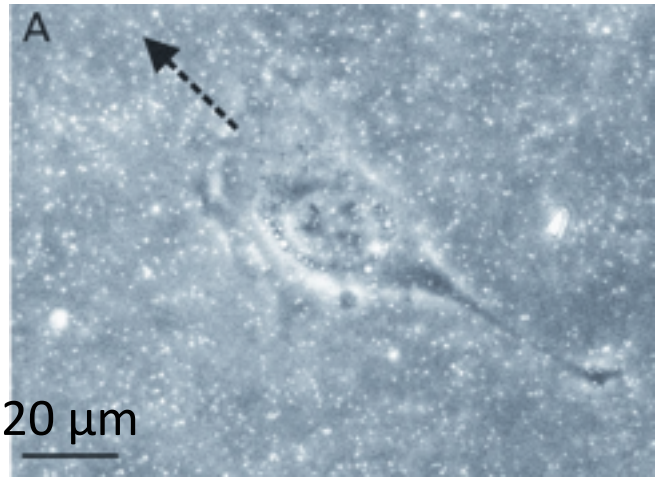
$$g_{13} = \frac{1+\upsilon}{2\pi E}\left(\frac{x_1 x_3}{r^3} - \frac{(1-2\upsilon)x_1}{r(r-x_3)}\right)$$

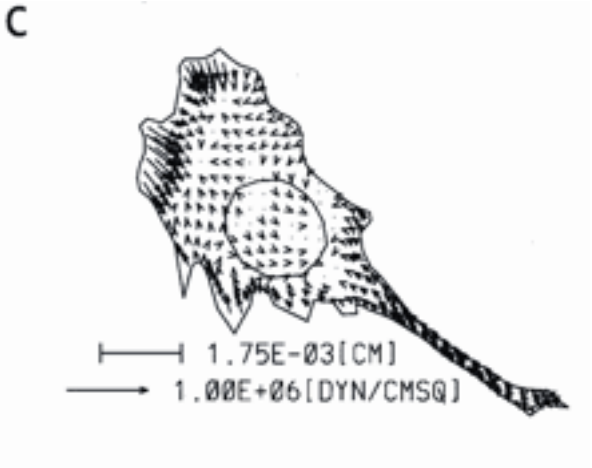
$$g_{23} = \frac{1+\upsilon}{2\pi E}\left(\frac{x_2 x_3}{r^3} - \frac{(1-2\upsilon)x_2}{r(r-x_3)}\right)$$

$$g_{33} = \frac{1+\upsilon}{2\pi E}\left(\frac{2(1-\upsilon)}{r} + \frac{x_3^2}{r^3}\right)$$

Dembo and Wang (1999) Biophys. J. 76:2307

Force measurements





Munevar et al., Biophys. J., 2001

E (Hydrogels)~10 kPa

$$u \sim \mu m$$

Markers embedded in a flexible substrate

↓

Force mapping

Inversion of the force-deformation relationship

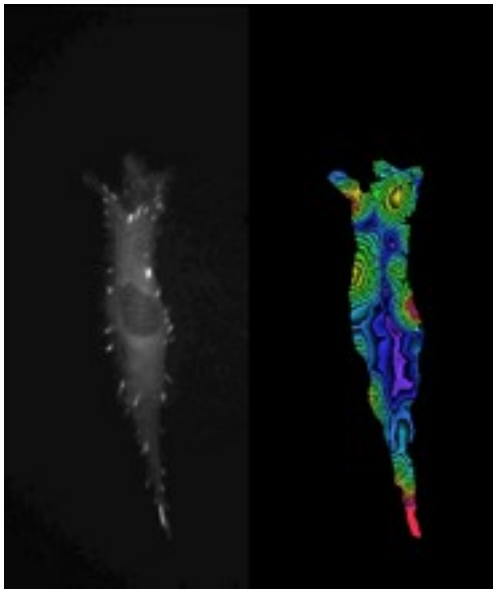
Dembo et al. Biophys. J. 1996; Butler et al. Am. J.Phys.-Cell Phys. 2002; Schwarz et al. Biophys. J. 2002; Barentin et al., Eur. Biophys. J. 2006

Color Mapping

Protrusion is Coupled to the Assembly of New Adhesions and Generation of Traction Forces at the Leading Edge

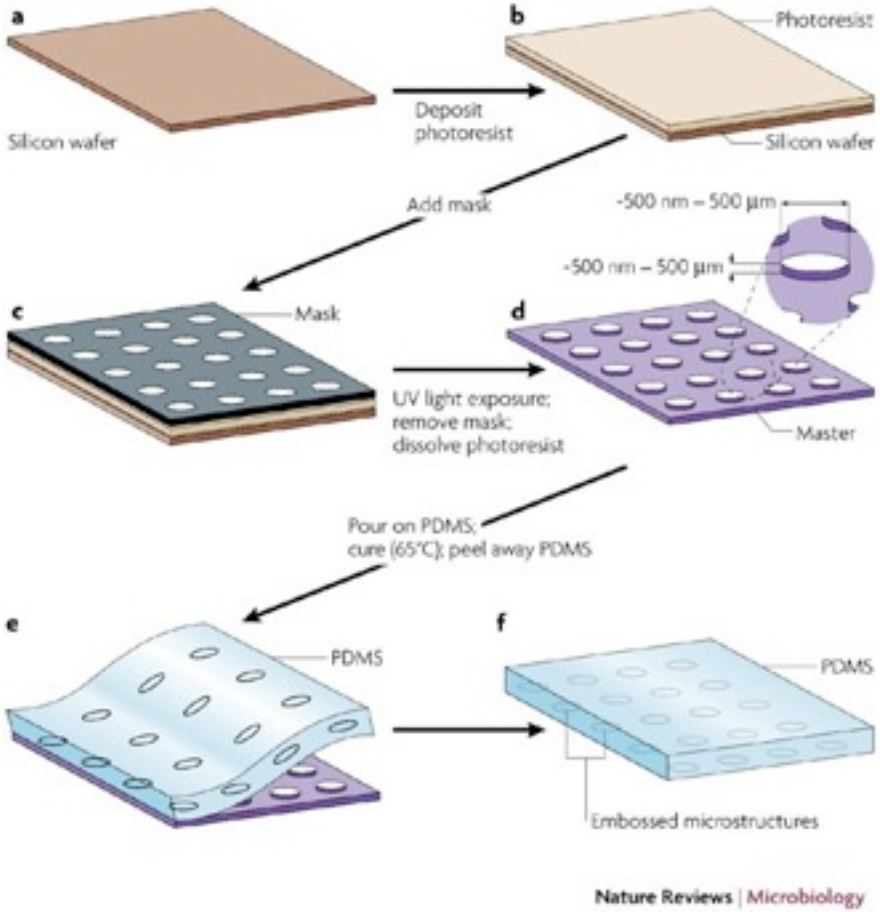
Yu-Li Wang's group

http://www.bme.cmu.edu/publications/yuliwang/index.html



How to control the micro-environment?

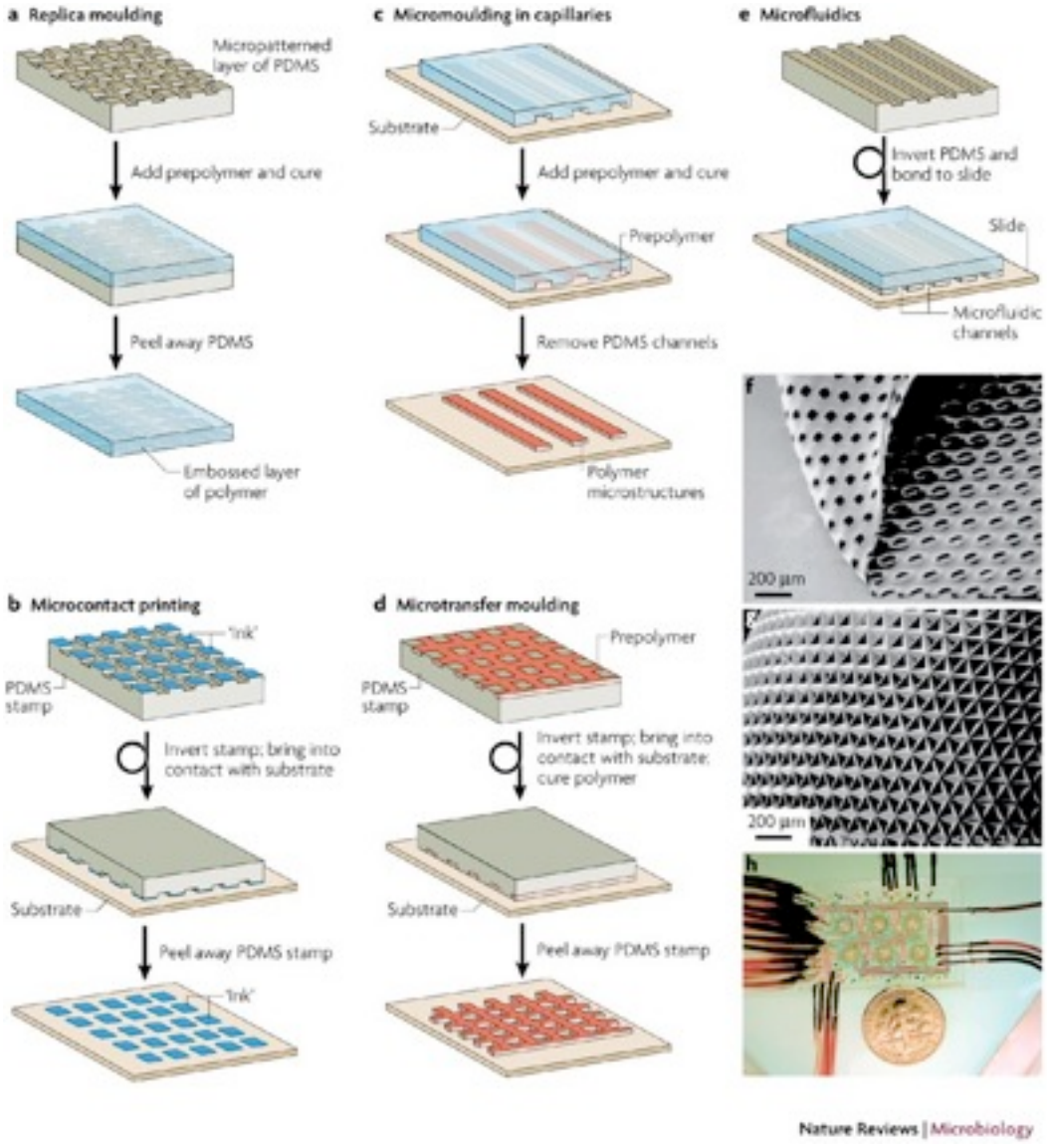
Soft lithography
(G. Whitesides, Harvard)



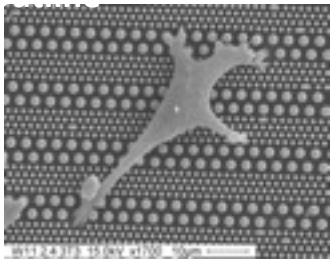
Weibel *et al. Nature Reviews Microbiology* 5, 209–218 (March 2007)

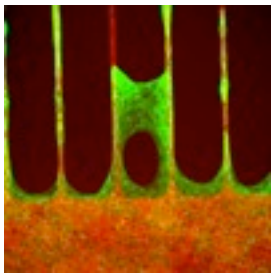
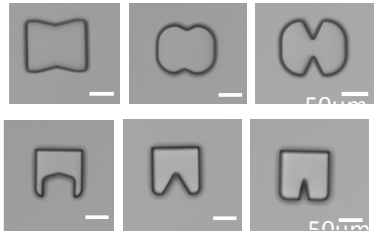
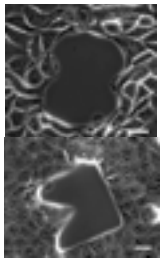
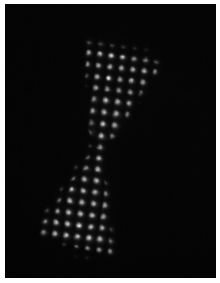
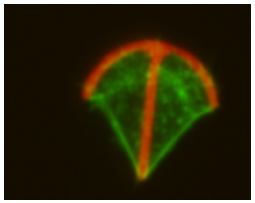
- Microfluidics
- Micro-stamping
- Micro-fabricated substrates

How to control the micro-environment?



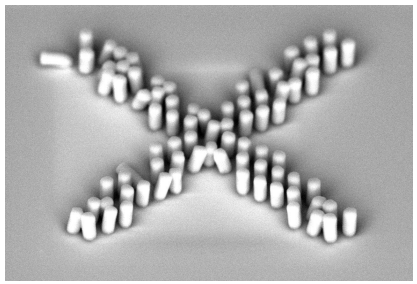
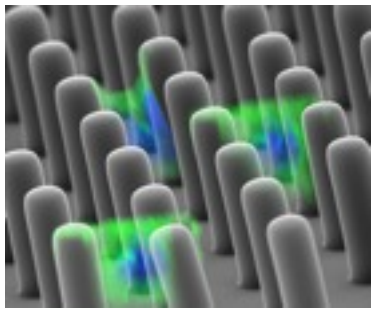
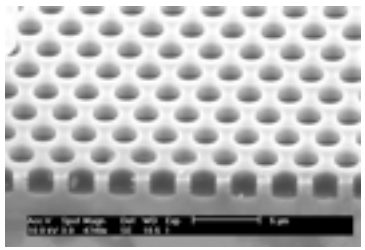
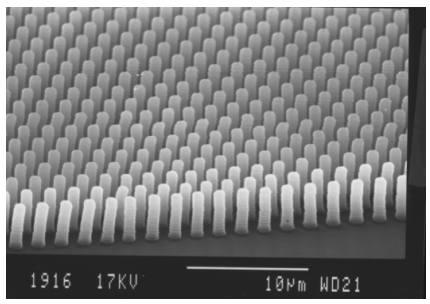
Weibel *et al. Nature Reviews Microbiology* 5, 209–218 (March 2007)

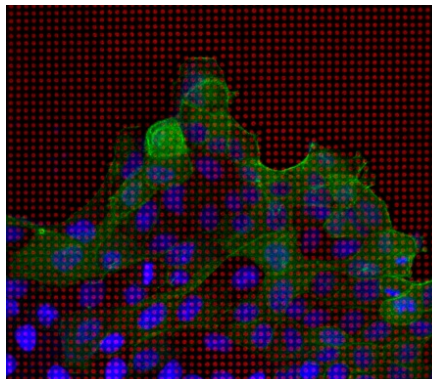
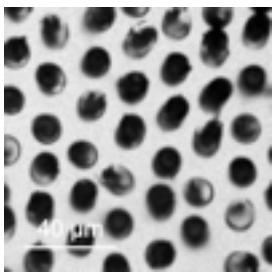
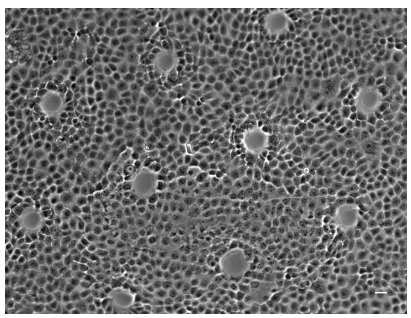
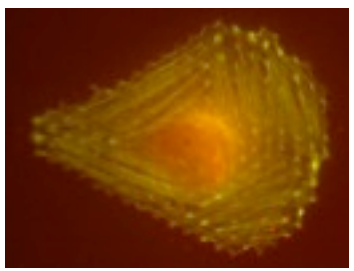


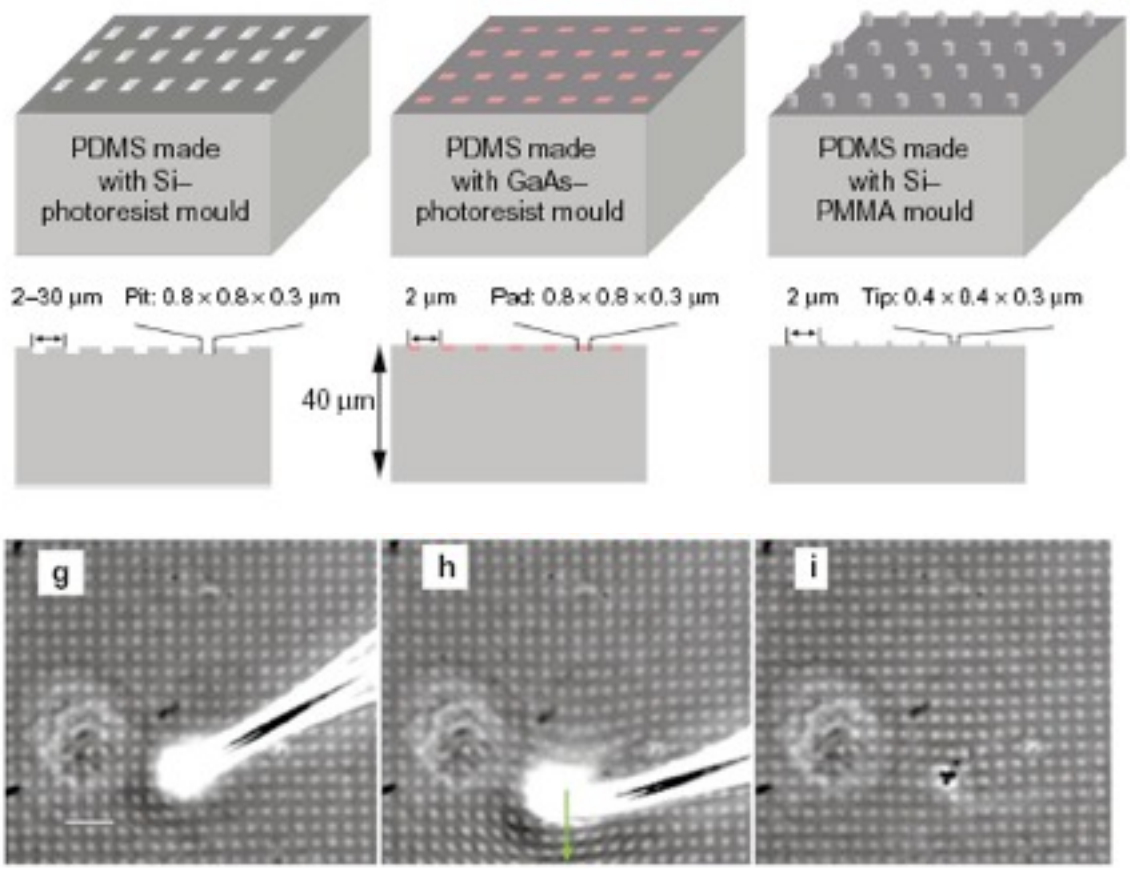
Microfabrication:

Systematic studies
&
Reproducible conditions

Microfabrication for force measurements
Adhesions and forces: Arrays of discrete deformation sensors



PDMS (poly di-methyl siloxane) = elastic gel of adjustable stiffness (varying crosslinking rate)

Microstructured substrate obtained by moulding. Each spot is used as a deformation sensor

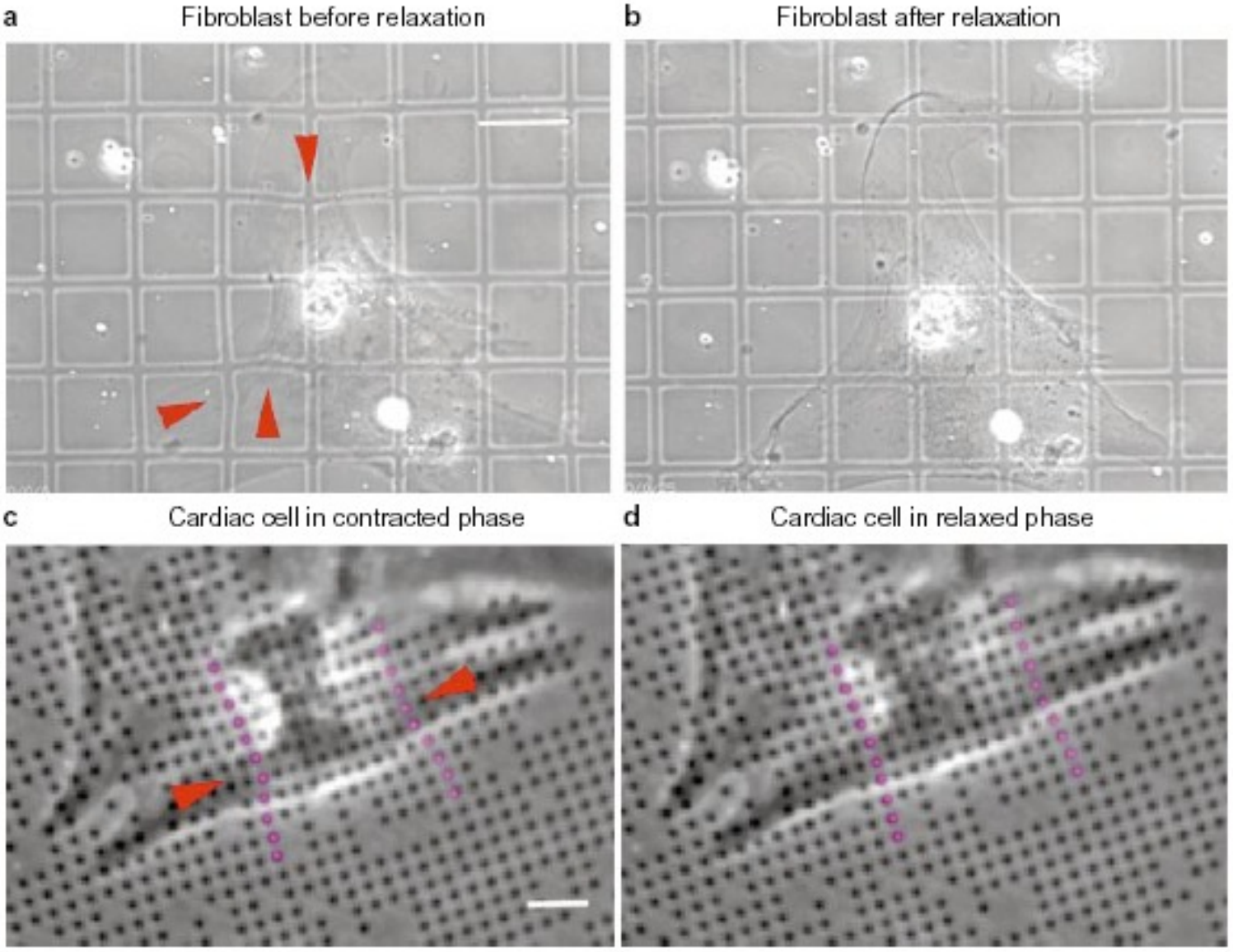
Deformation assay with a micropipette

Balaban et al. Nature Cell Biol. 3, p 466 (2001)

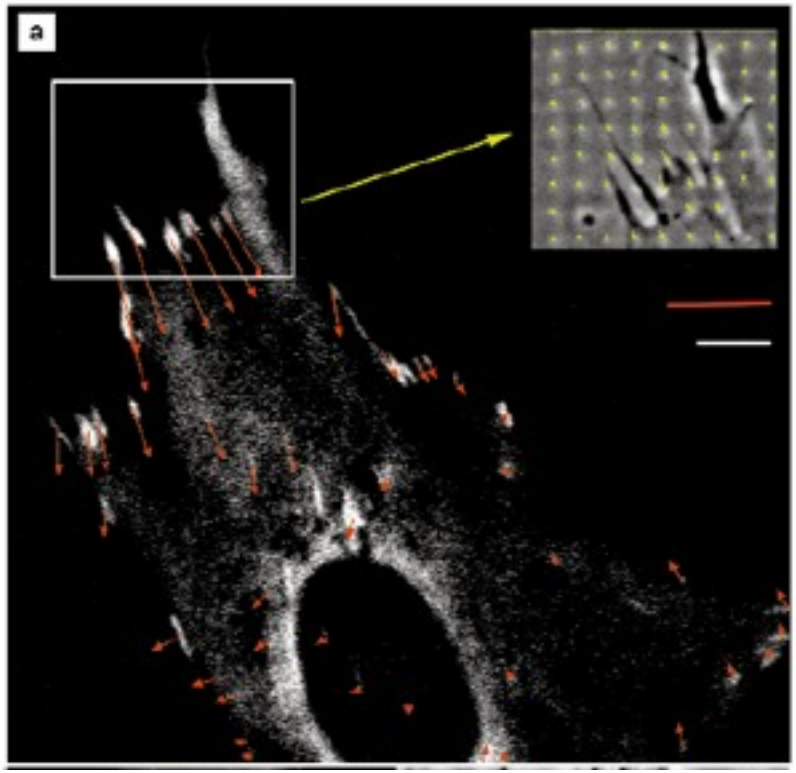
Again…Convert deformations into stress requires numerical treatment

Examples of deformation recording

BDM added = inhibits myosin activity and cell contractility

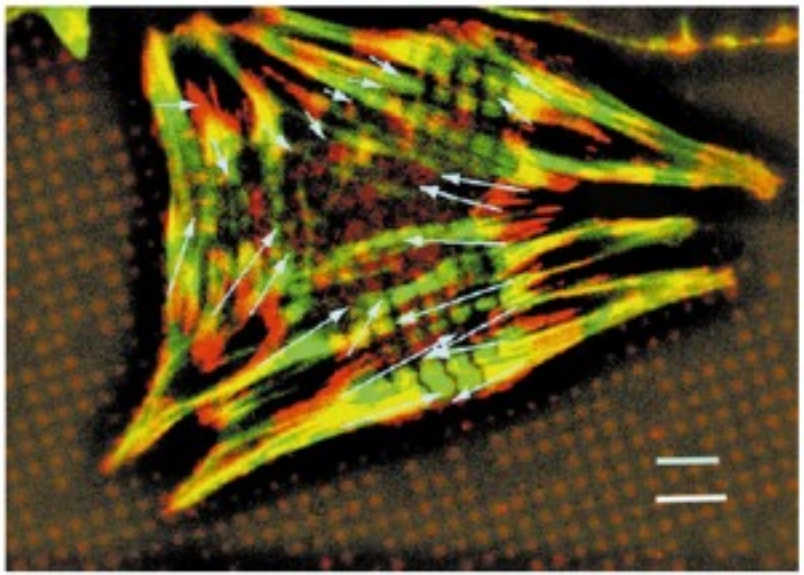


Dynamics of focal adhesions



Fibroblast labelled with GFP-vinculin
plated on a patterned substrate

The forces exerted on the substrate are parallel to the elongation of focal adhesions

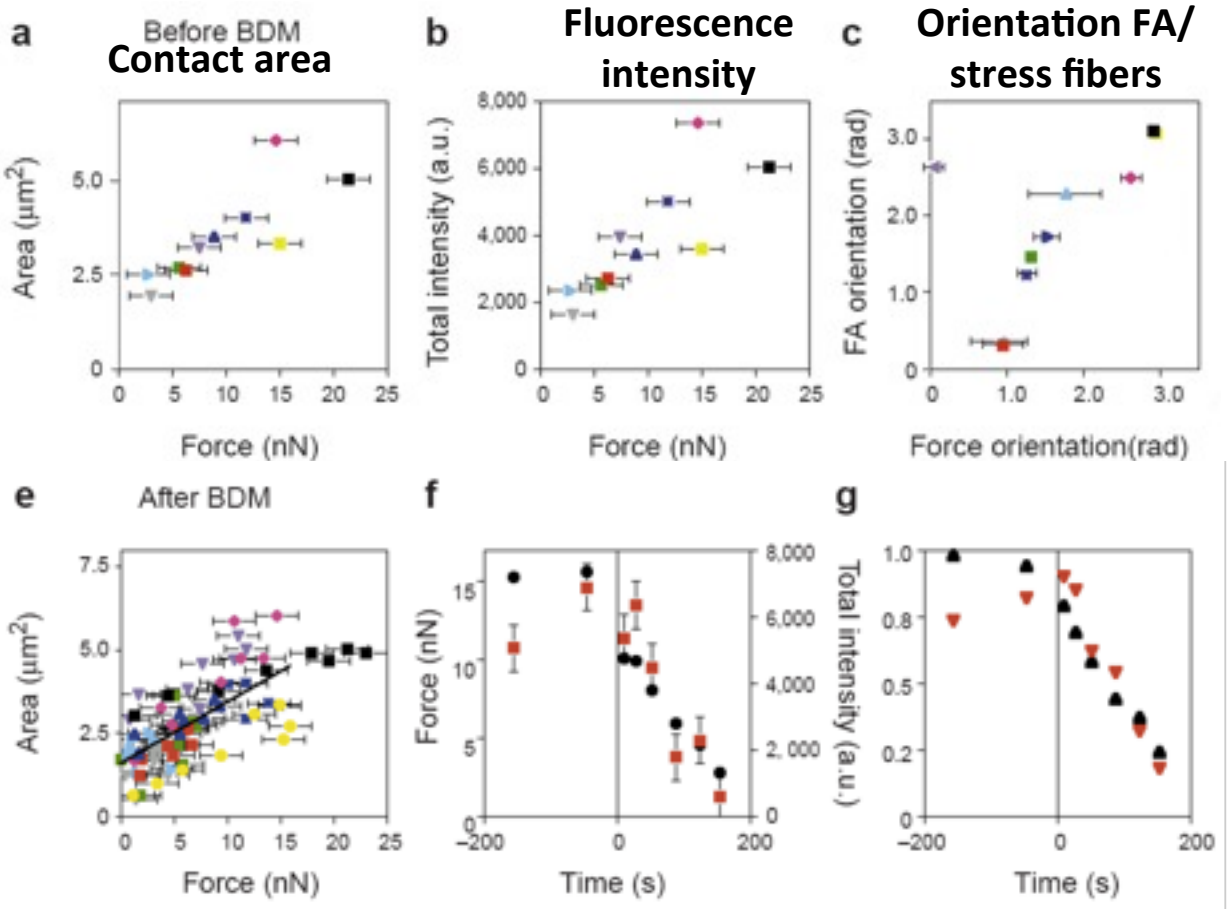


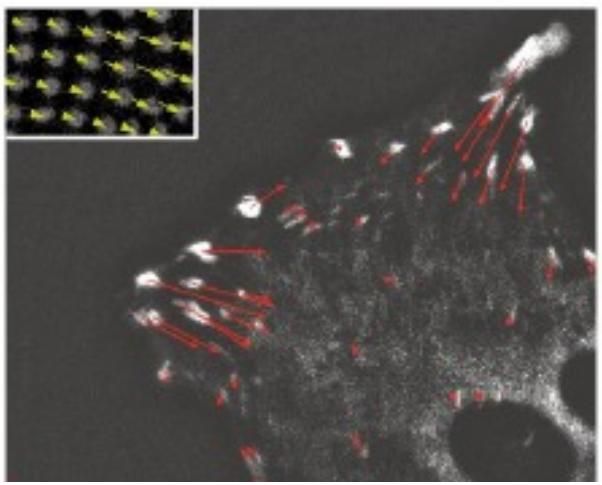
Cardiac myocyte labelled with GFP-vinculin (red) and actin (green).

Exerted forces are parallel to stress fibers

Balaban et al. Nature Cell Biol. 3, p 466 (2001)

Stress applied through focal adhesions and myosin activity in contact strength





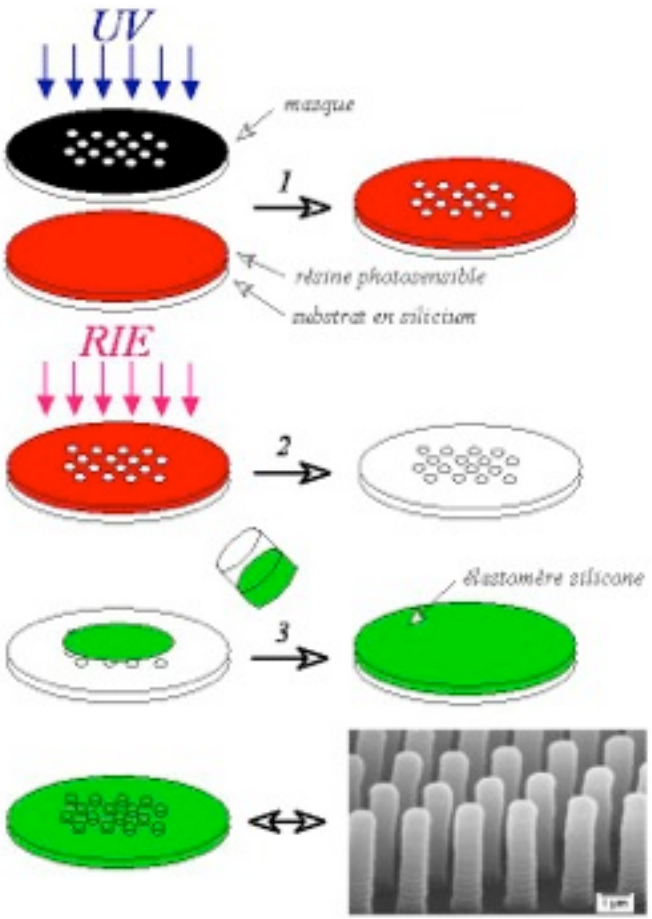
A t= 0, addition of BDM (butanedione monoxime) = inhibitor of myosin ATPase activity

FA areas as a function of forces
Slope=Stress is constant :5.5 nN/μm²

Microfabricated substrates

Micropillars (Rovensky et al. Exp. Cell Res. 1991 ; Tan et al., PNAS 2003; Roos et al., ChemPhysChem 2003; du Roure et al., PNAS 2005)

Different approach: *combination of « hard » and « soft » lithography (GM. Whitesides, Harvard)*

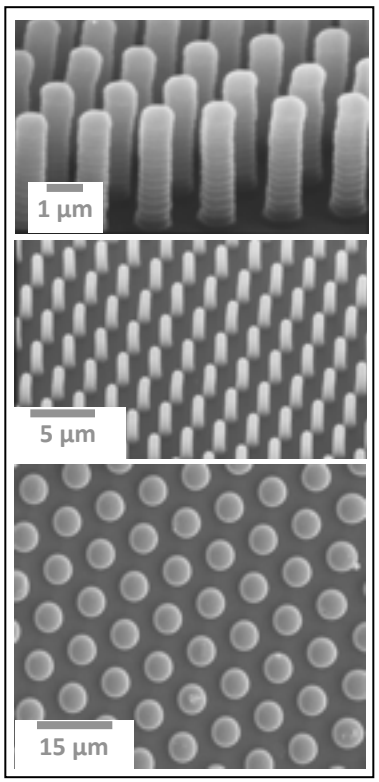


Advantages of PDMS :

Biocompatibility

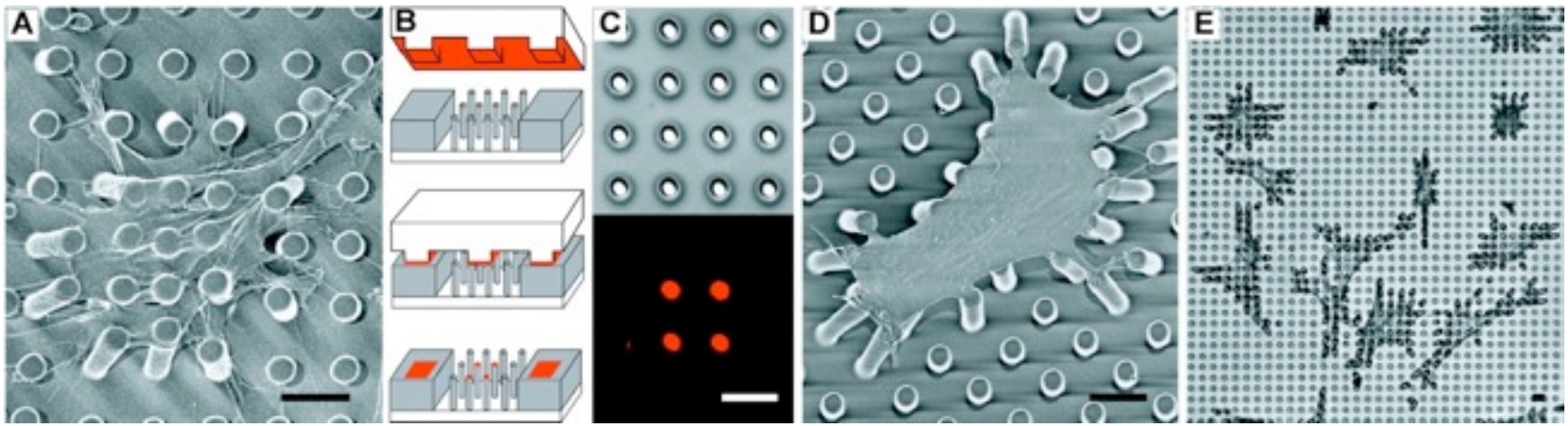
Optically transparent

Possibility of surface treatment



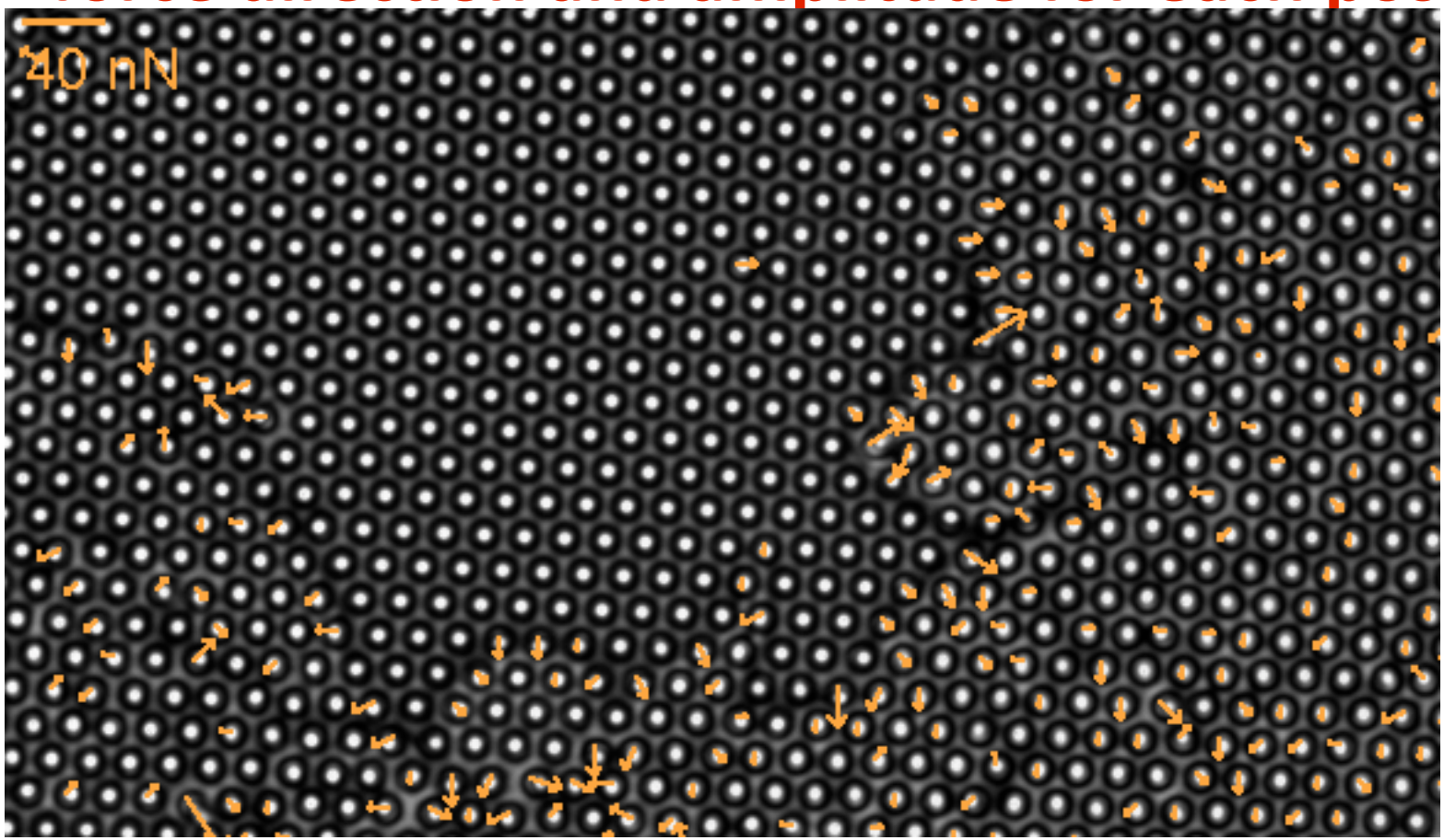
Microfabrication : great versatility in size

Cell culture on the substrate and deformations



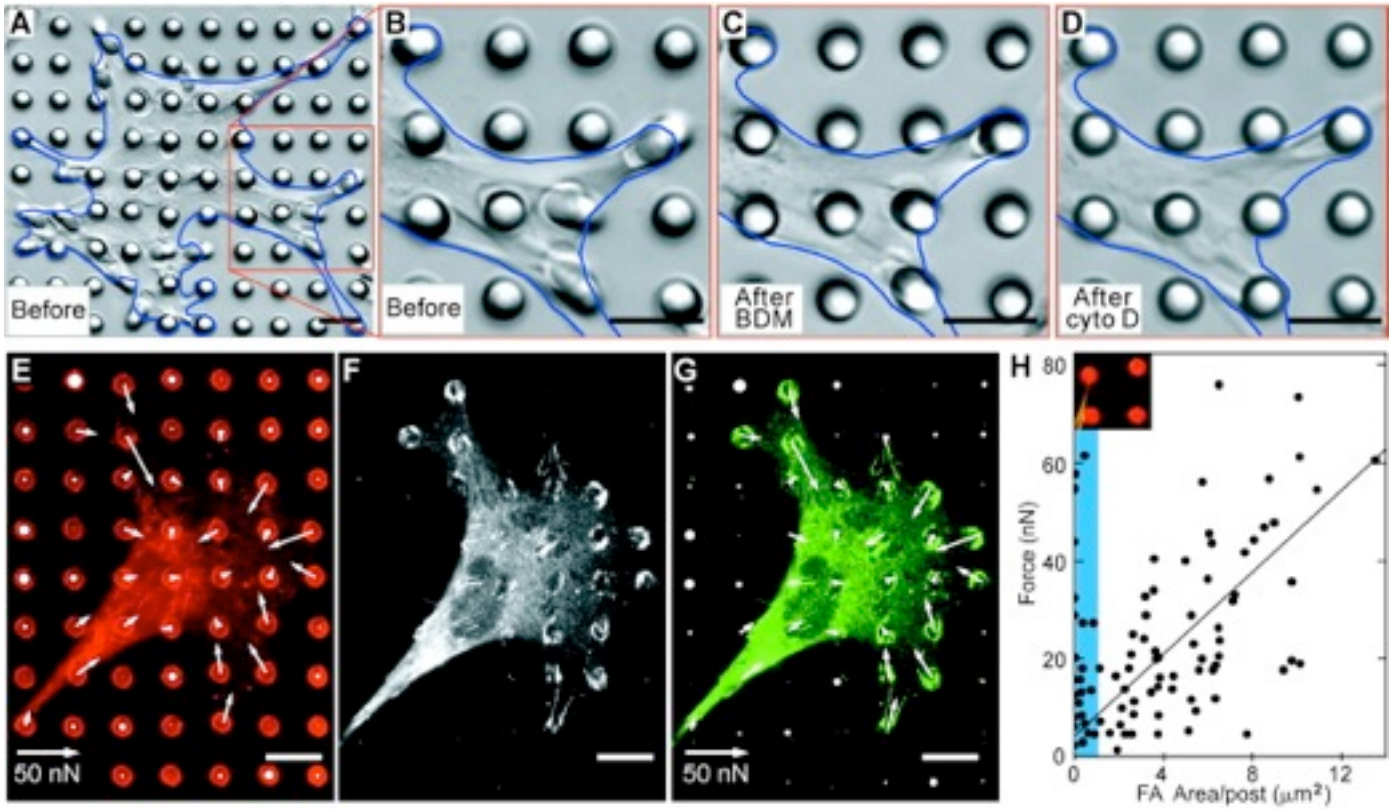
Tan, John L. et al. (2003) Proc. Natl. Acad. Sci. USA 100, 1484-1489

Vector field on epithelium edges : force direction and amplitude for each post



(du Roure *et al.*, PNAS, 2005)

Traction forces and focal adhesions

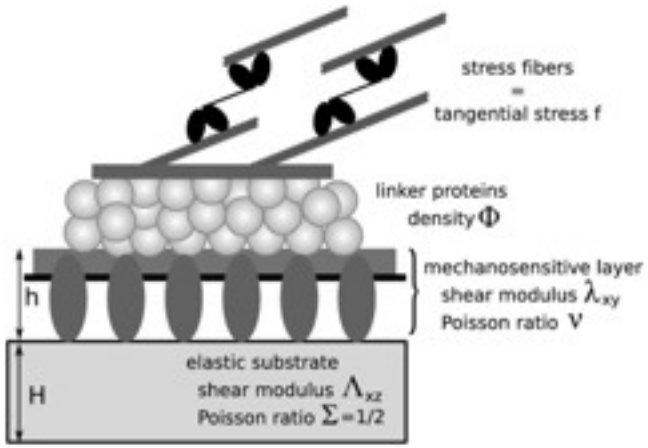


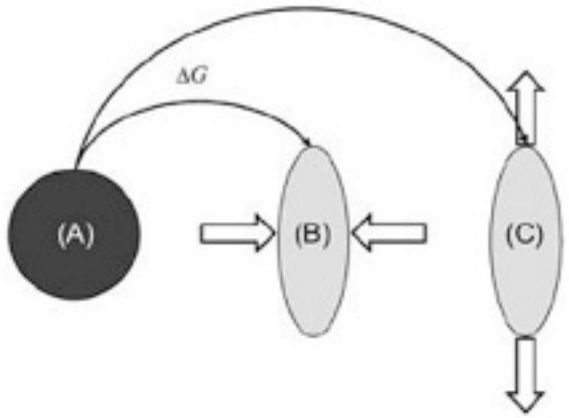
- FA areas as a function of forces: Good agreement with previous studies
- Constant stress ≈5.5 nN/μm²
- High forces observed for small adhesive contacts

Tan et al. (2003) Proc. Natl. Acad. Sci.

A model for the contact growth: Local mechanosensitivity

Nicolas et al., PNAS 2004; Besser and Safran, Biophys J, 2006; Nicolas et al., Biophys. J. 2006

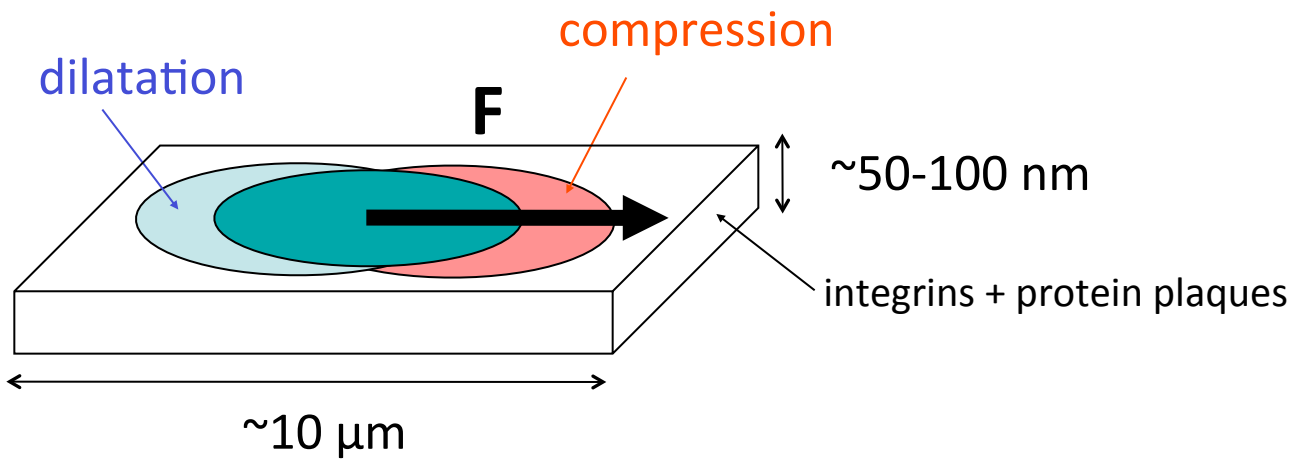




Some proteins (integrins ?) of the contact are force sensitive : they may change their conformation under stress (in compression or in extension), which makes possible the recruitment of other plaque proteins (and of actin filaments)

FAs = autonomous mechanosensor implies that the stress on FA is regulated by the FA itself!

Elastic model



Infinite elastic solid subjected to a local stress ⇨ anisotropic deformation

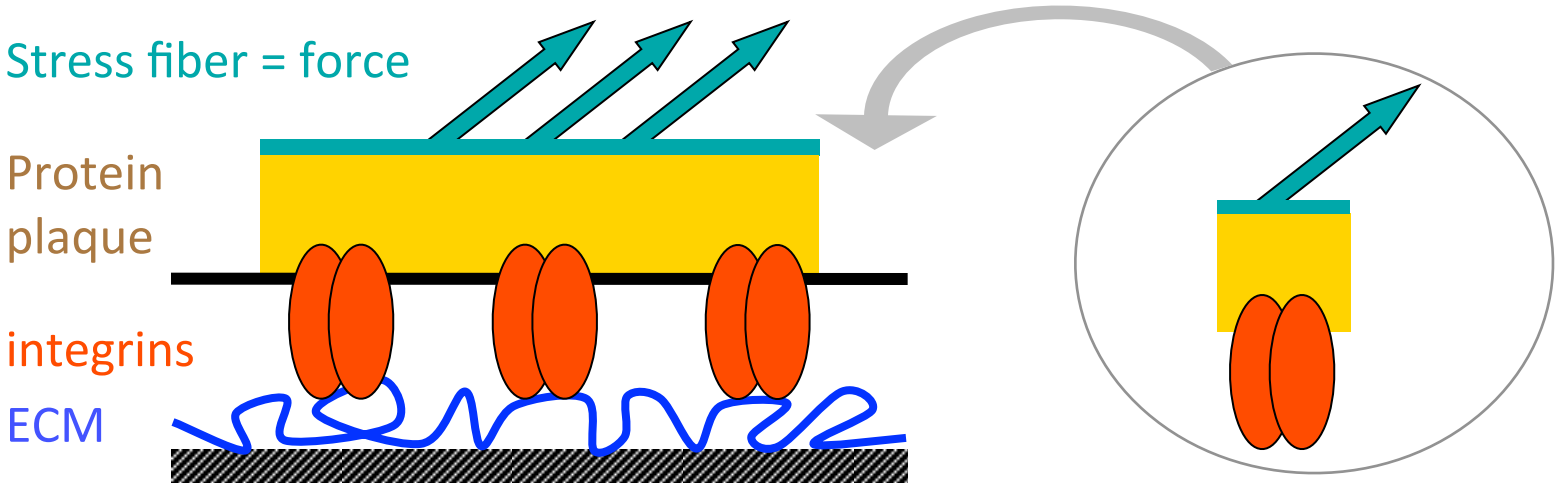
Assumption :

Local force on the adhesion

⇨ Biochemical response related to the deformation

Adhesion growth = adding a new particule

Modeling by Nicolas & Safran



Work of a larger number of stress fibers (+)

Chemical energy associated by the absorption of new molecules (-)

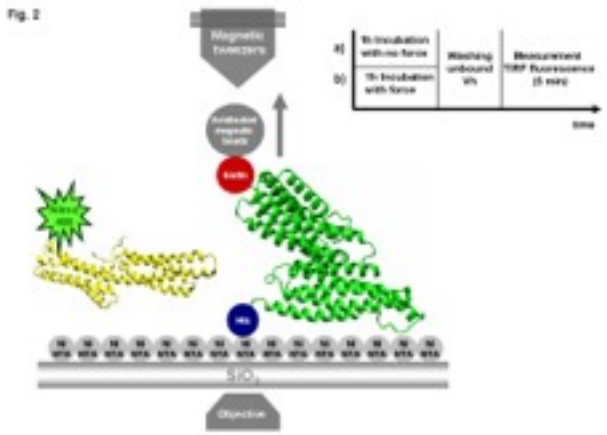
- Anisotropic contact growth in the direction of the force
- Local regulation by the stress

A. Nicolas et al. PNAS 2004; Biophys J. 2006

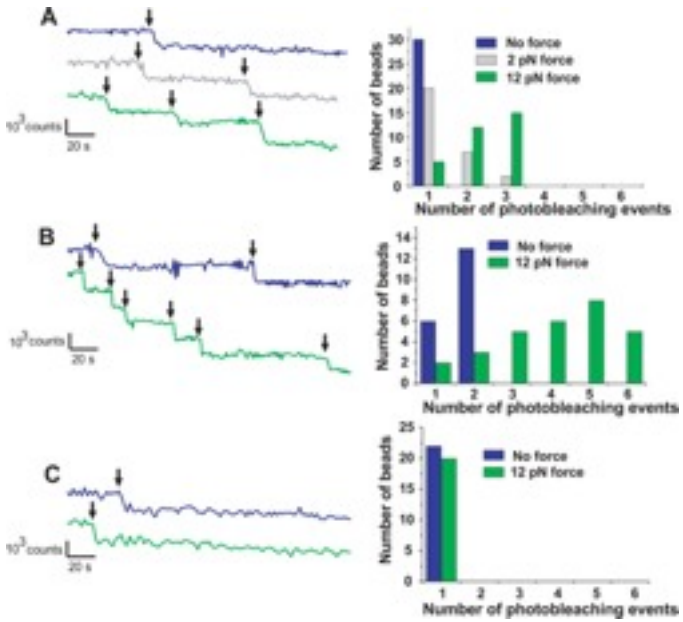
Local mechanosensors ?

➢Protein unfolding? (del Rio et al., Science 2009)

Stretching of an individual talin rod exposing cryptic sites for binding the focal adhesion molecule, Vinculin



Influence of forces



➢ Ion channels?

Response of the adhesion to stress is triggered by the opening of ion channels: (Glogauer et al. J Cell Sci. 1997)

Ion channels: Polarized response of FAs to force?

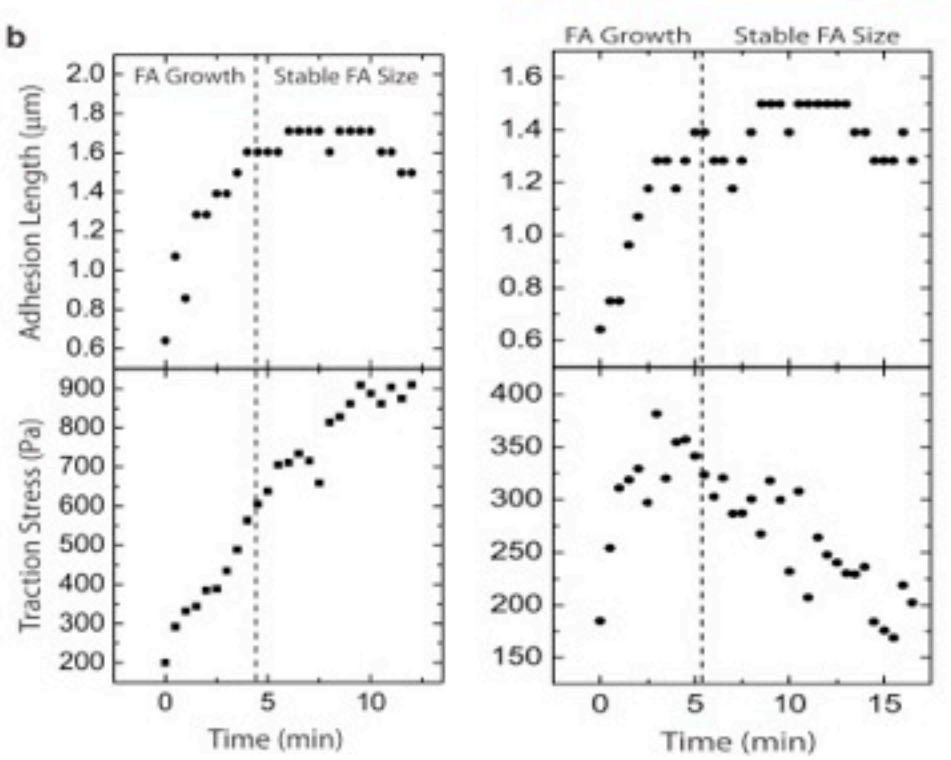
Diffusion of such small ions as calcium very fast :D 10^{-10} to 10^{-12} m^2/s

Isotropic distribution in a very short time

Ion channels cannot be the initiators of the response of FAs to force but may stabilize FAs

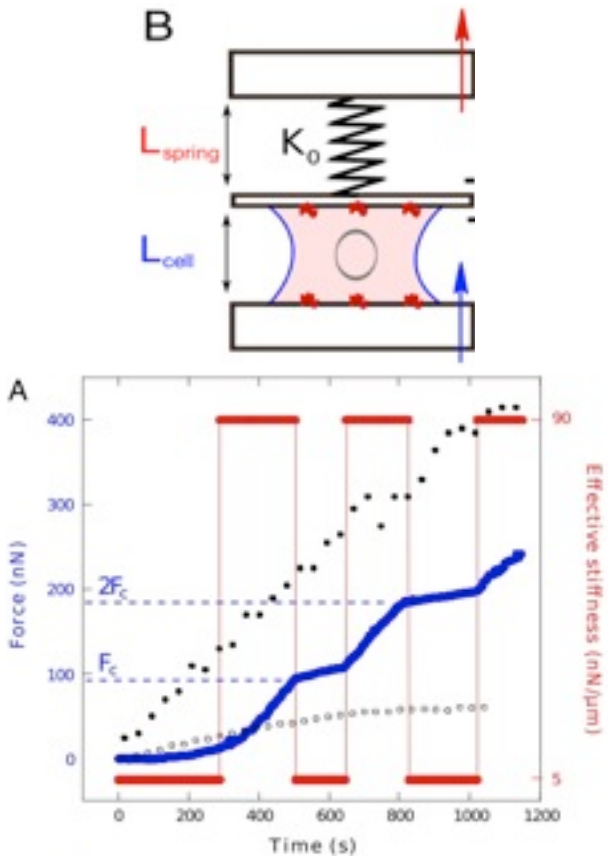
But...

Focal Adhesion stress is not constant



(Stricker et al 2011)

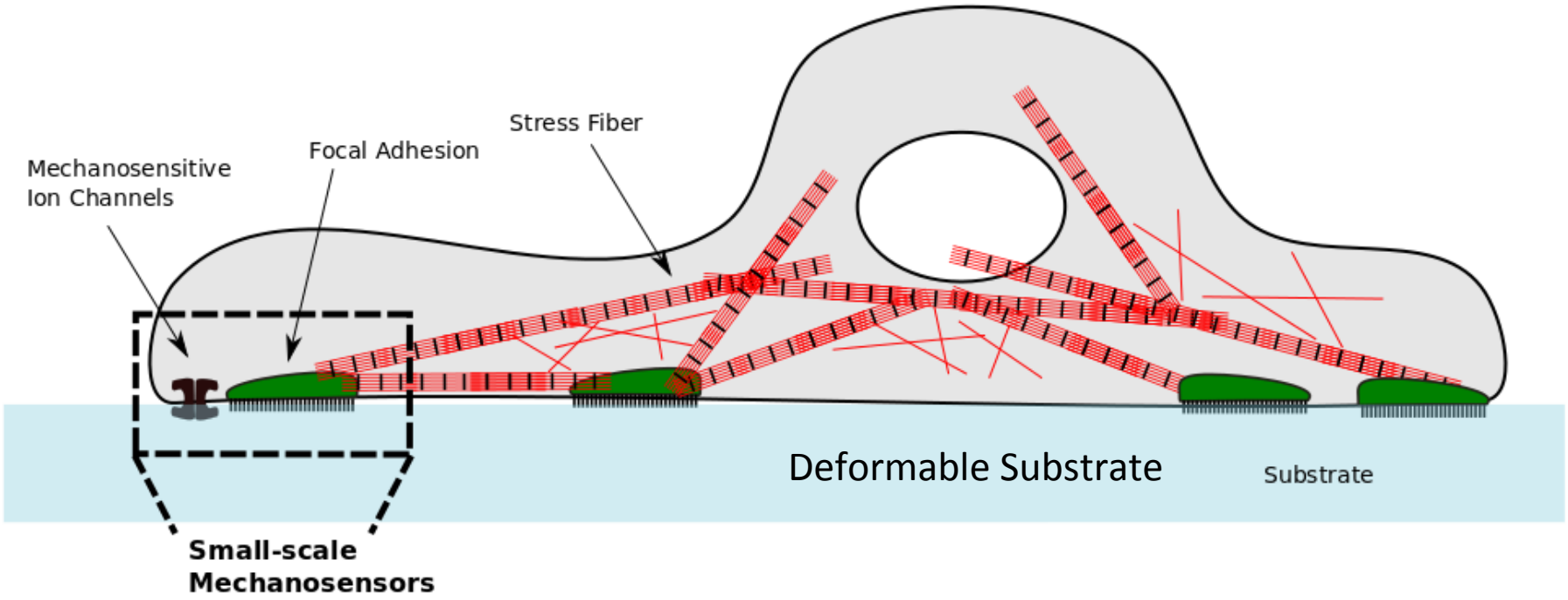
Cells respond to mechanical forces in seconds



(Mitrossilis et al 2010)

Bio-chemical signaling cascades cannot explain response times of ~0.1s

Rigidity sensing mechanisms

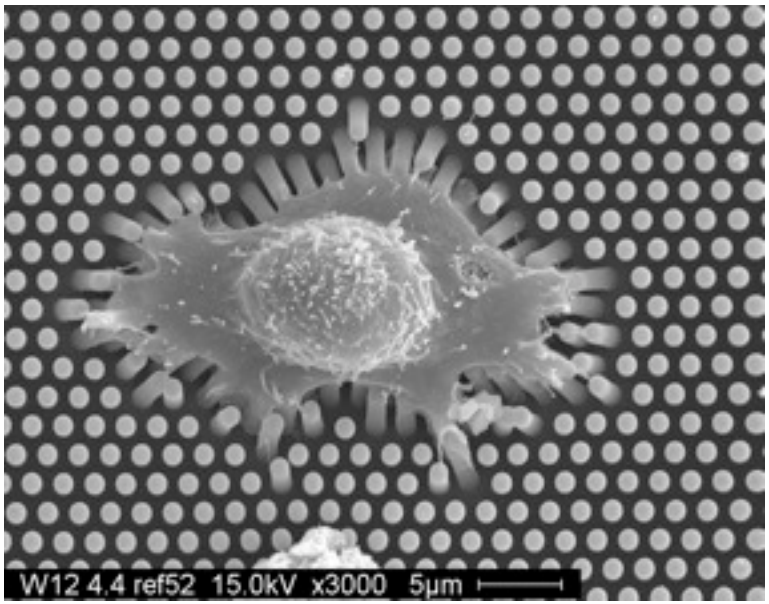


- FAs respond to rigidity: local scales
(Pelham et al. PNAS 1997; Balaban et al. NCB 2001; Del Rio et al. Science 2009; Stricker et al. Biophys J. 2011; Plotnikov et al. Cell 2012...)

- Acto-myosin cytoskeleton / Mechanosensitivity: larger scales
(Mitrossilis et al. PNAS 2009; Crow et al. 2012)

Micropillar substrates

A dense array of µ-pillars

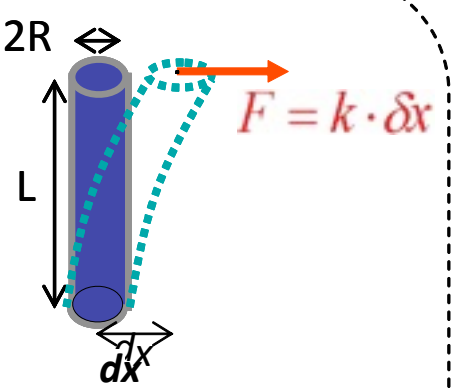


10 000 000 cantilevers/cm^2

Spring constant

$$k = \frac{3}{4}\pi E \frac{R^4}{L^3}$$

Young's modulus



E= 2 MPa

R= 1 or 2 µm

L=1 up to 8 µm

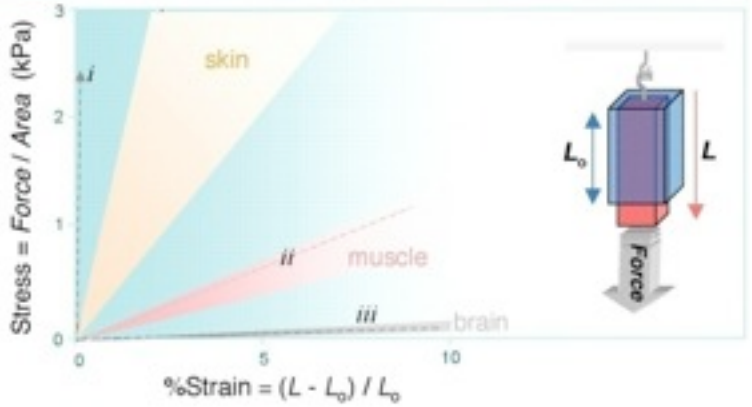
k= 1.5 up to 200 nN/µm

- Measurements of traction forces (du Roure et al. PNAS 2005)
- Large range of stiffnesses

In vivo environmental stiffness

In vivo

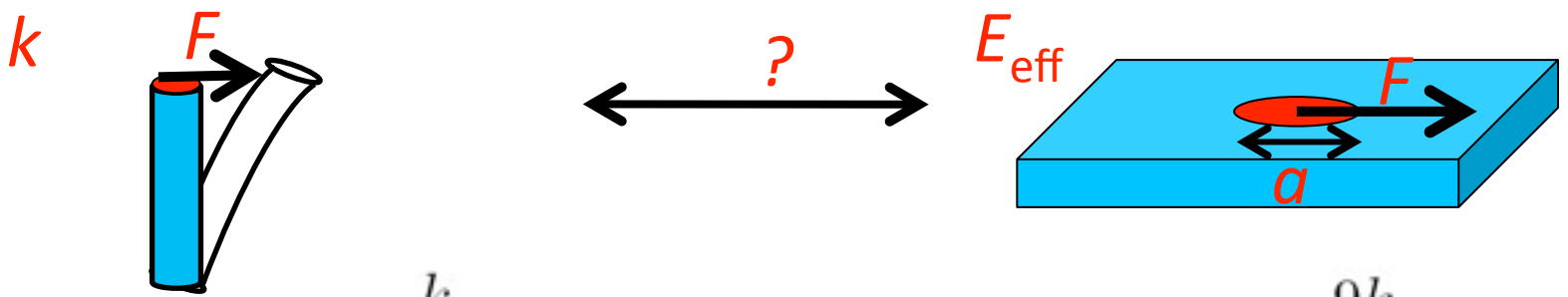
$$E = 1 \text{ to } 100 \text{ kPa}$$



D. E. Discher et al., Science 310, 1139 -1143 (2005)

In vitro

Micropillar versus continuous substrate



$$E_{\text{eff}} \propto \frac{k}{a}$$

a: adhesion size

$$E_{\text{eff}} = \frac{9k}{4\pi a}$$

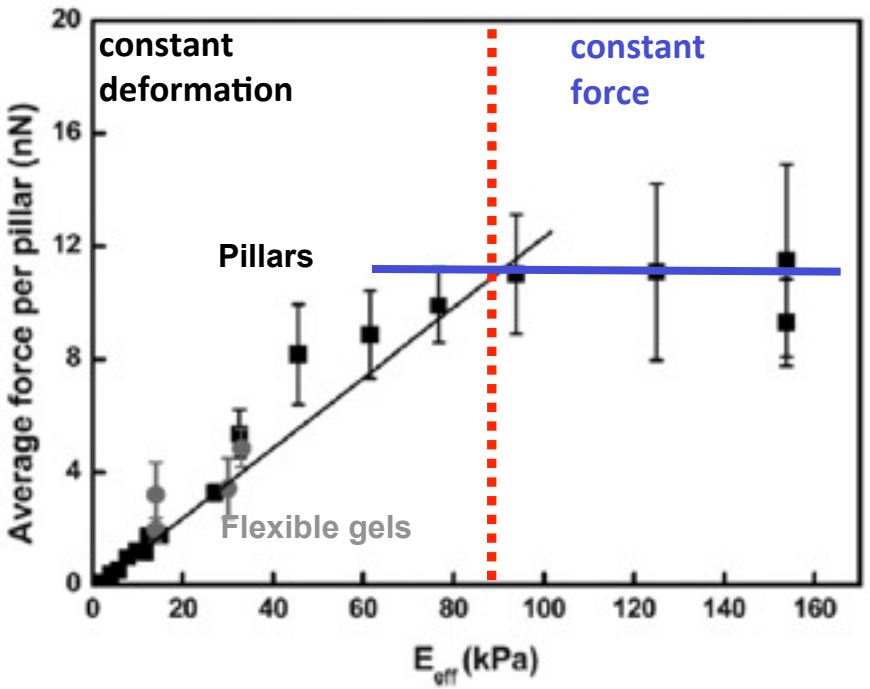
$$k \approx 1 \text{ to } 200 \text{ nN}/\mu\text{m}$$
$$a \approx 1 \ \mu\text{m}$$

$$E_{\text{eff}} = 0.7 \text{ to } 140 \text{ kPa}$$

Ghibaudo *et al.*, Soft Matter, 2008

Force-rigidity relationship

3T3 fibroblasts



Two regimes :

$E < E_{threshold}$: Force adaptation to the rigidity
Displacement ≈ 130 nm

$E > E_{threshold}$: Force saturation : 11 nN

Good agreement with previous experiments on continuous flexible gels (Yu-Li Wang's group; Lo et al. Biophys. J. 2000)

Linear relation between F and K ($\propto E_{eff}$)

Consequences

$F = k \cdot \delta x$

$F = Cte \cdot k$

$\delta x = Cte \approx$ 150 - 200 nm

Regulation by strain or stress ?

Theoretical model by Nicolas & Safran
(Nicolas et al.,PNAS 2004; Biophys J, 2006)

Balaban *et al*. , Nat Cell Biol, (2001) :

- Force — Area of focal adhesions
- Stress (σ)=F/Area of FAs=CST

Local or global mechanism?

Regulation by strain or stress?

Plausible explanation:
Active matter modeling (P. Marcq et al. Biophys. J. 2011)

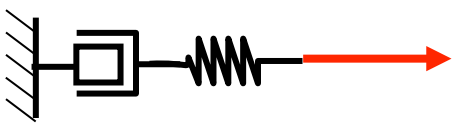
- Coarse-grained models of the actin cytoskeleton / active matter theory (activity of molecular motors) (Kruse et al. EPJE 2005)

- polar viscoelastic gels

- Dynamics is given by Navier-Stokes equation

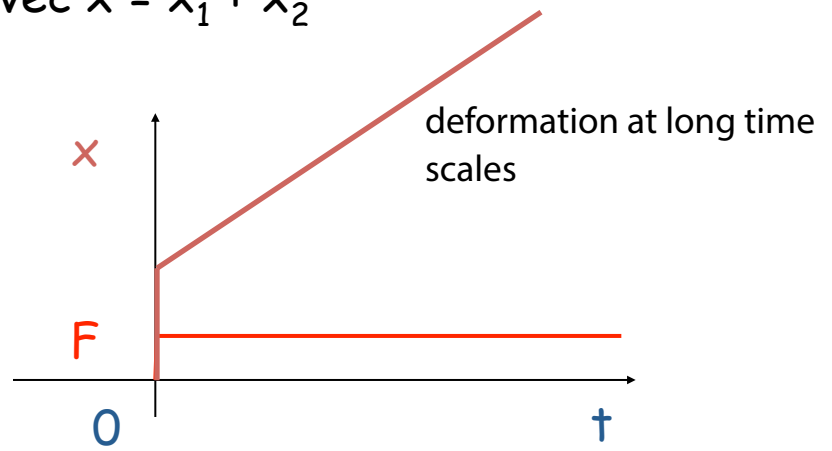
- Active stress (activity of motors / ATP hydrolysis)

Example : Maxwell liquid



$F = kx_1 = \gamma \dot{x}_2$ avec $x = x_1 + x_2$

$$\frac{dx}{dt} = \frac{1}{k}\frac{dF}{dt} + \frac{F}{\gamma}$$



Response to a force step : Creep function J(t)

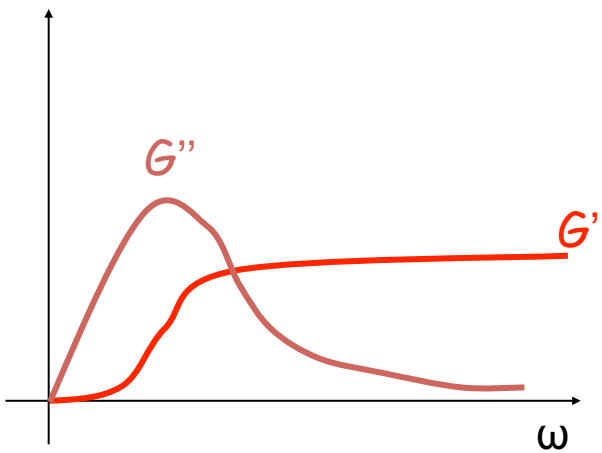
$$J(t) = \frac{x(t)}{F} = \left(\frac{1}{k} + \frac{t}{\gamma}\right)$$

Response to a sinusoidal force : Viscoelastic Modulus G(ω)

$F = F_0 \exp(j\omega t)$

$x = x_0 \exp(j\omega t)$, x_0 complex

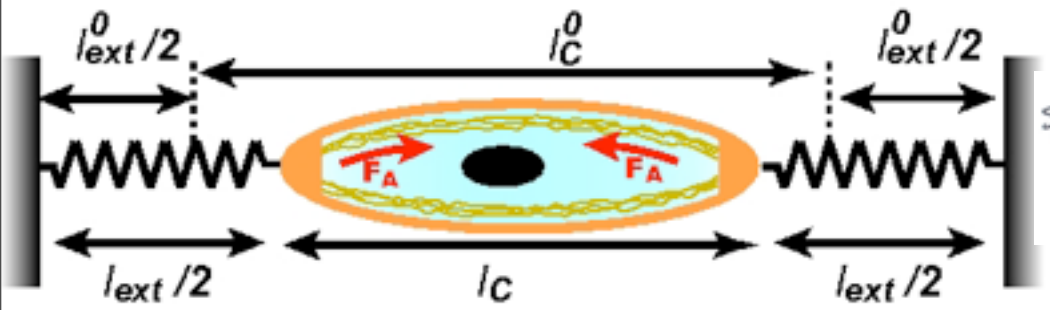
$F_0 = G(\omega)\, x_0$



$$x_0 = \left(\frac{1}{k} + \frac{1}{i\gamma\omega}\right) F_0 \qquad G(\omega) = \frac{k\gamma^2\omega^2}{k^2 + \gamma^2\omega^2} + i\frac{k^2\gamma\omega}{k^2 + \gamma^2\omega^2}$$

Rigidity sensing explained by active matter theory

Philippe Marcq, Natsuhiko Yoshinaga and Jacques Prost



Stress : $\sigma = \underbrace{\sigma^{el}}_{\text{passive (here, elastic)}} + \underbrace{\sigma_A}_{\text{Active}}$ $\left(\sigma_A \propto \Delta\mu\right)$

Linear regime : 1) elasticity, spring, $\ell_c(t)$, k_c

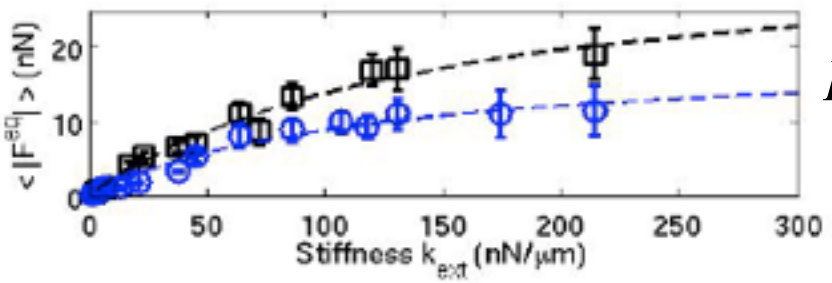
2) activity, $F_A = \sigma_A S$ $(F_A < 0)$

- External environment (pillar) : $F(t) = -k_{ext}\left(\ell_{ext}(t) - \ell^0_{ext}\right)$ (exerted by the cell on the environment)
- $\ell_{tot} = cte = \ell_c(t) + \ell_{ext}(t)$
- No inertia : $F(t) = k_c\left(\ell_c(t) - \ell^0_c\right) + F_A$

$$F^{eq} = f_{sat}\,\frac{k_{ext}}{k_{ext} + k_c}$$

if $k_{ext} \ll k_c$

$$f_{eq} \simeq \left(\frac{f_{sat}}{k_c}\right) k_{ext}$$



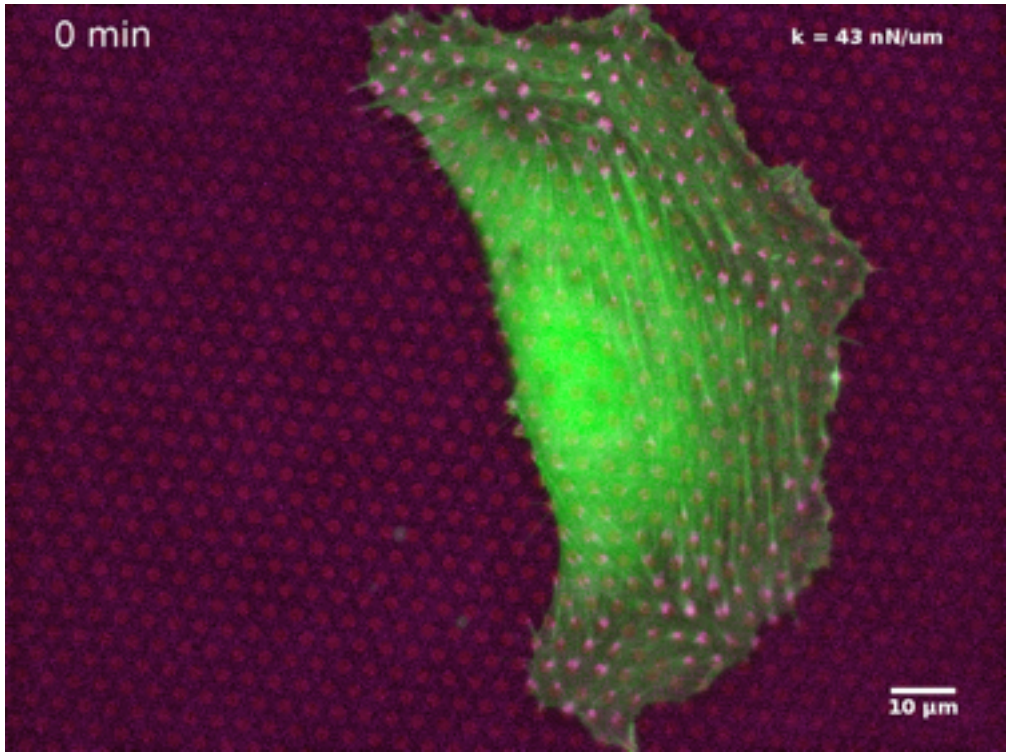
$$F_{Sat} \approx 10nN$$

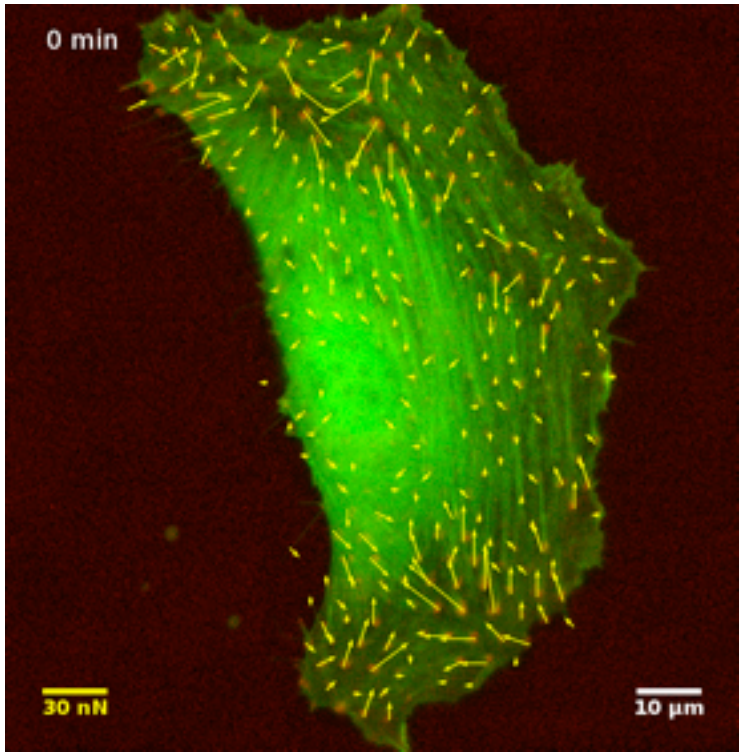
$$k_c \sim 10^2\, nN/\mu m$$

$$E \sim k_c\, d/S \sim 10^5\, Pa$$

Correlation between traction forces, actin and FAs

REF 52 cell
paxillin pink
actin green
pillar red

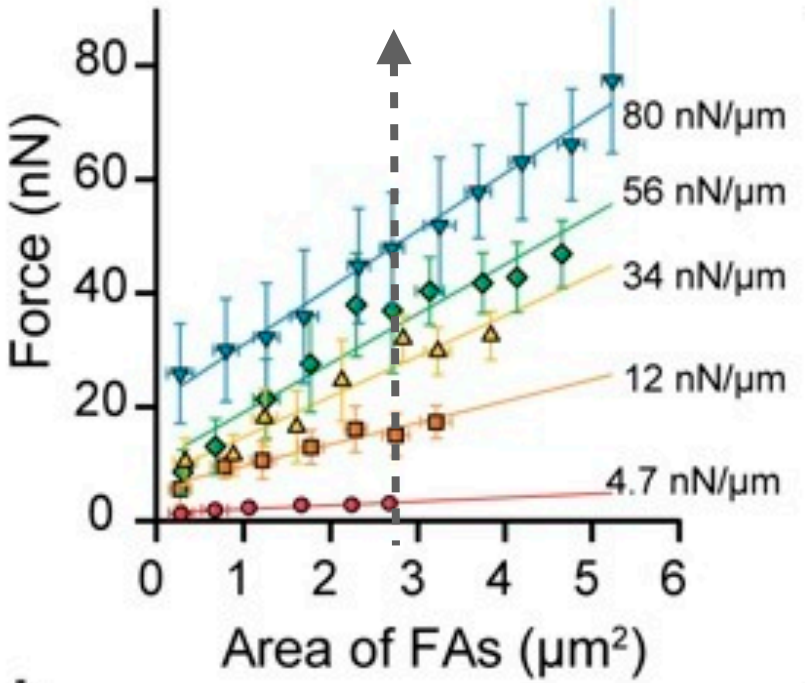




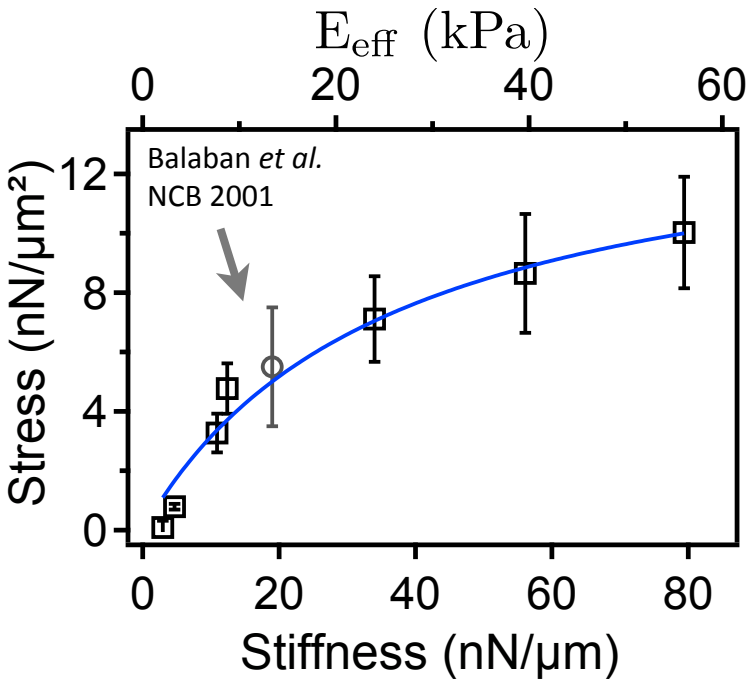
- Relationship between FAs and traction forces
- Relationship between actin organization and traction forces

Stress-stiffness relationship

Same size but different forces



Variations of the stress

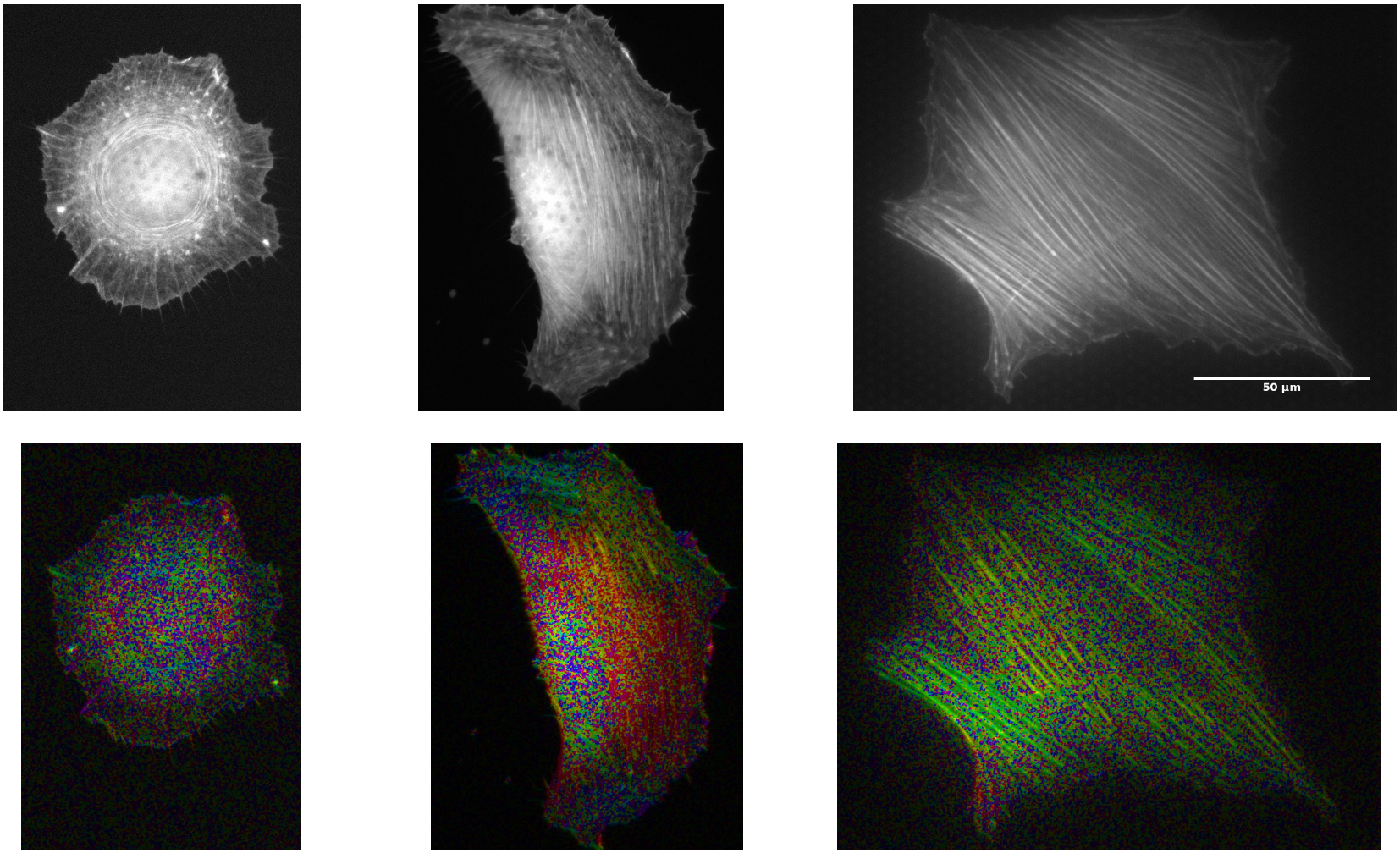


Trichet et al. PNAS 2012

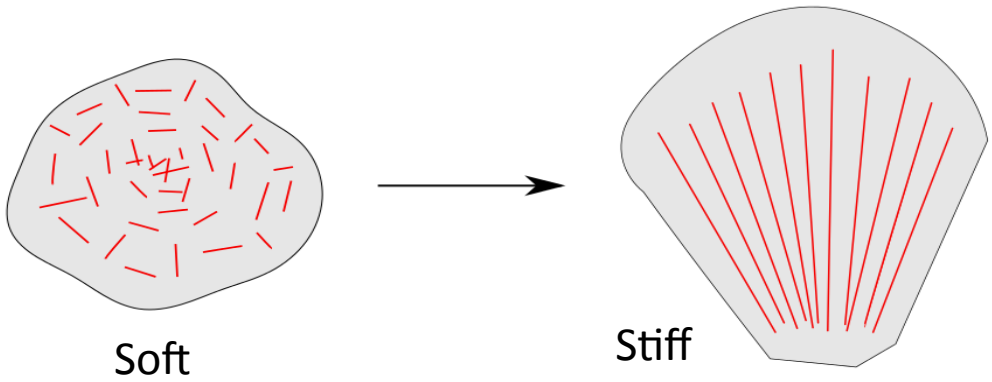
- Stress (Force/FAs Area) varies with the rigidity
- Two regimes: Fast increase up to 30 nN/μm and saturation above 40 nN/μm
- Regulatory mechanosensing process ?

Actin polarization in response to substrate stiffness

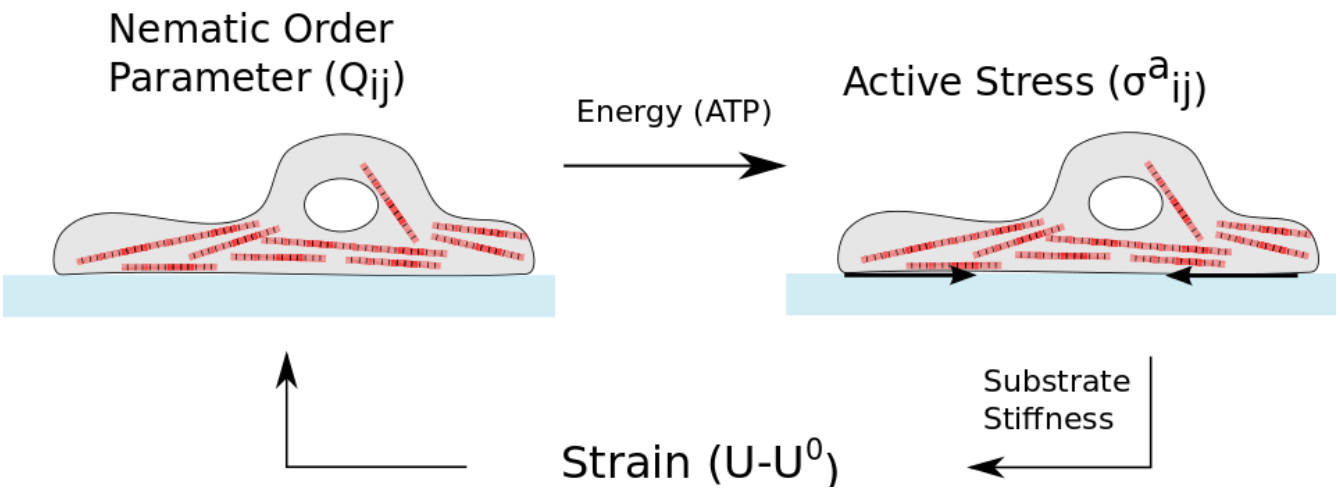
Mukund Gupta



Large-scale mechanosensitive response

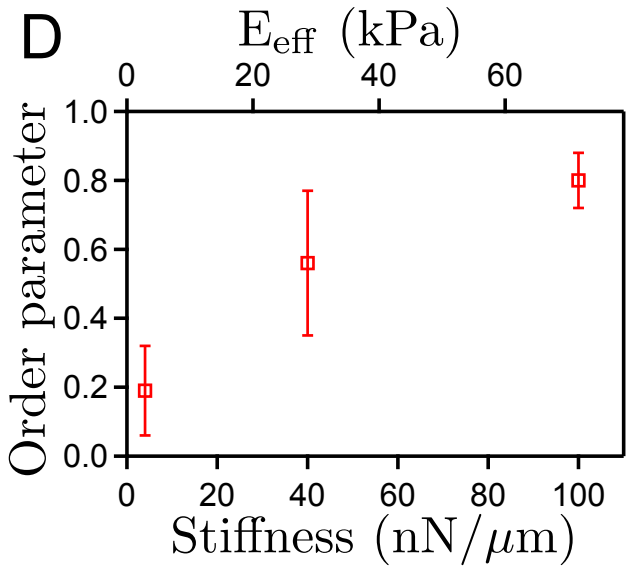


actin cytoskeleton behaves like an active polar gel:



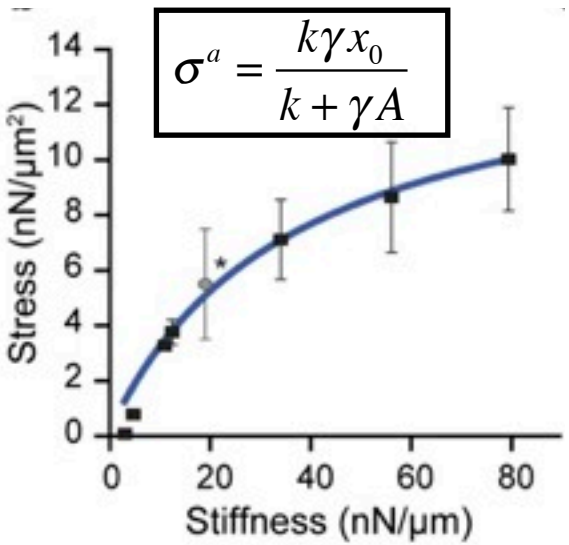
Large-scale mechanosensing

Order parameter of stress fiber orientation as a function of substrate stiffness

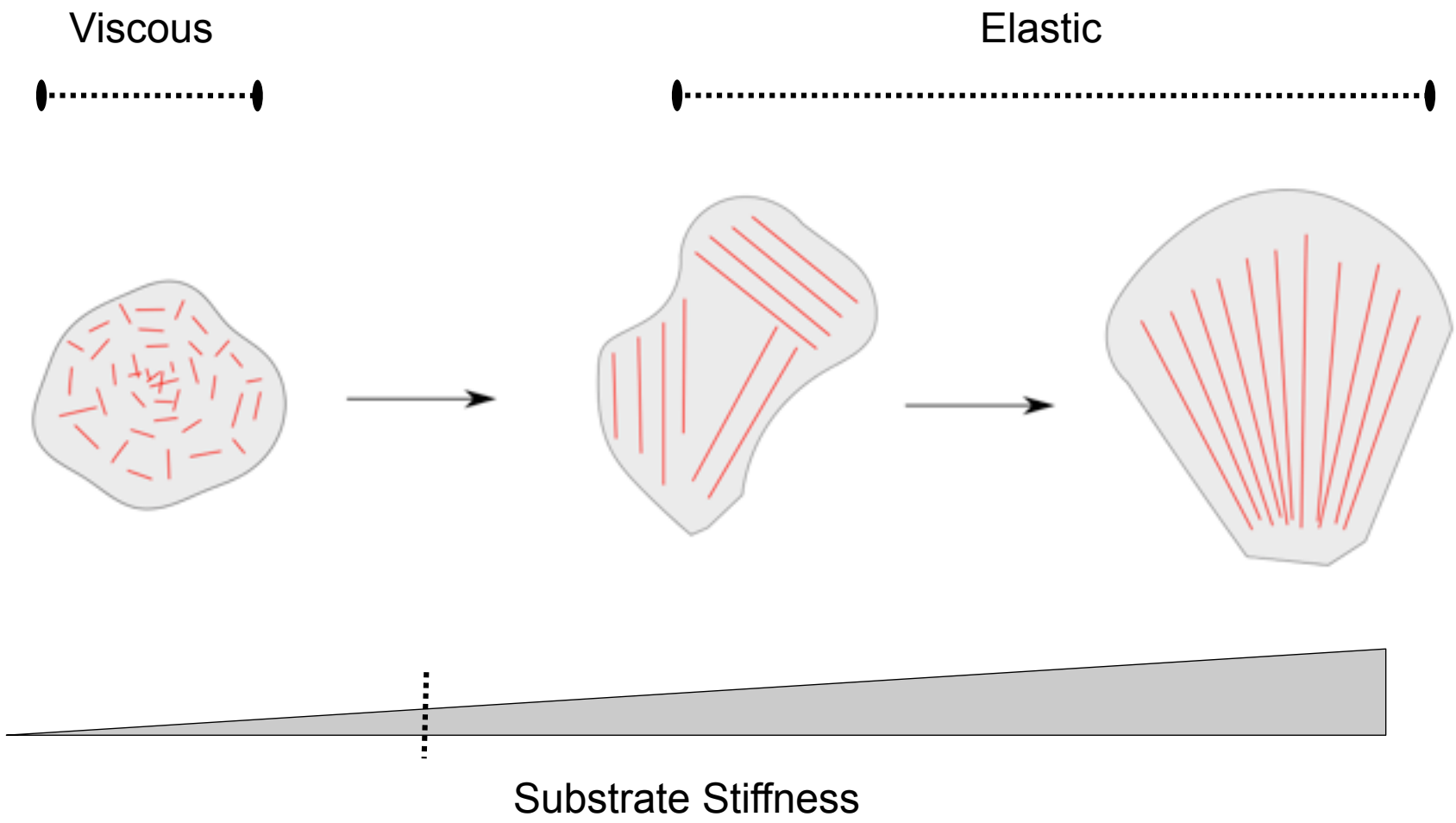


Linear coupling between the stress, σ^a & the strain, x
(similar to Zemel *et al.* Nat Phys. 2010, Marcq et al. Biophys. J. 2011)

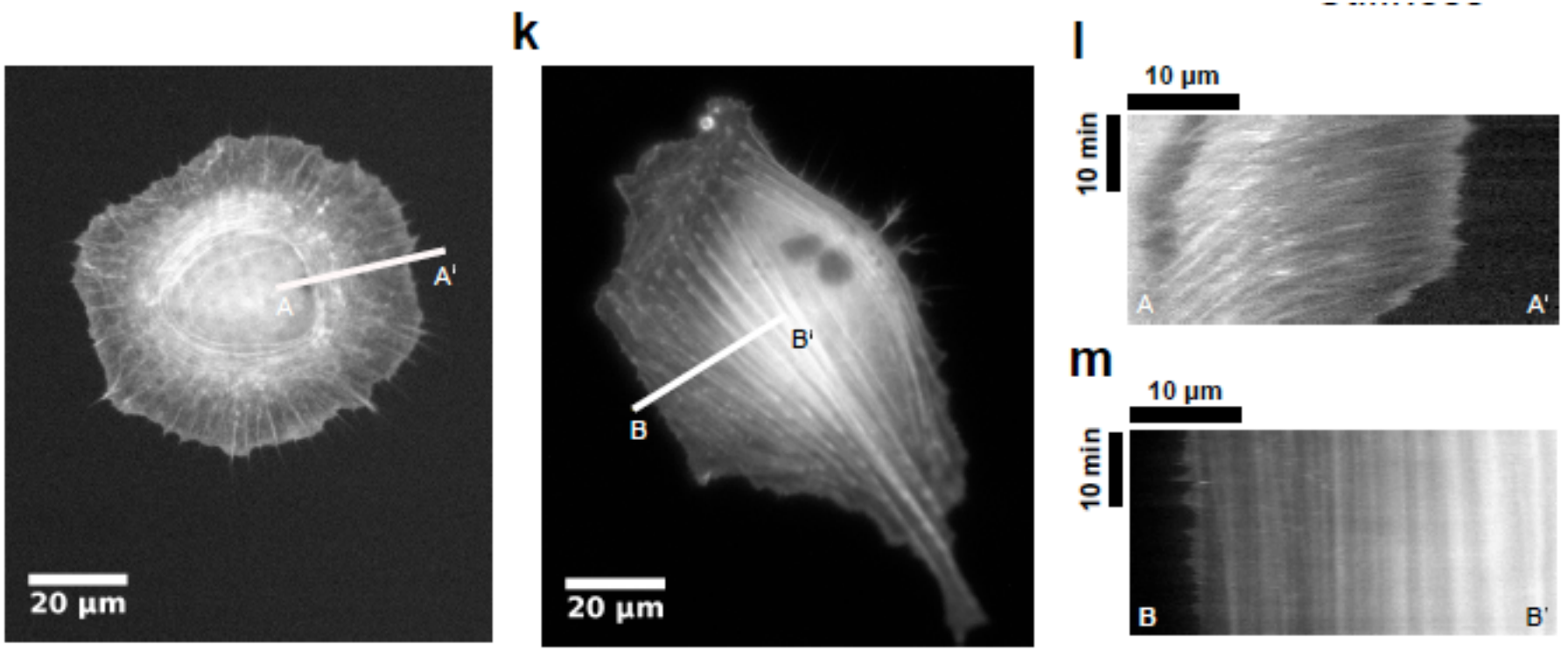
For 1D stress fiber: $\sigma^a = -\gamma.(x-x_0)$



Viscoelastic behavior of actin cytoskeleton



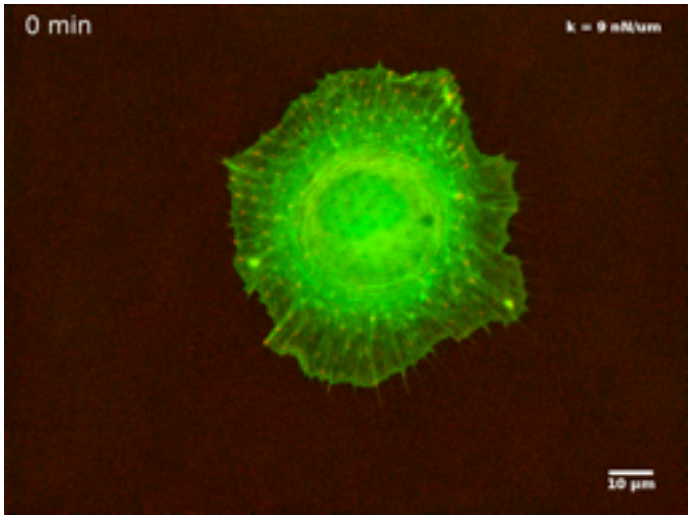
Elastic *versus* viscous behaviors?



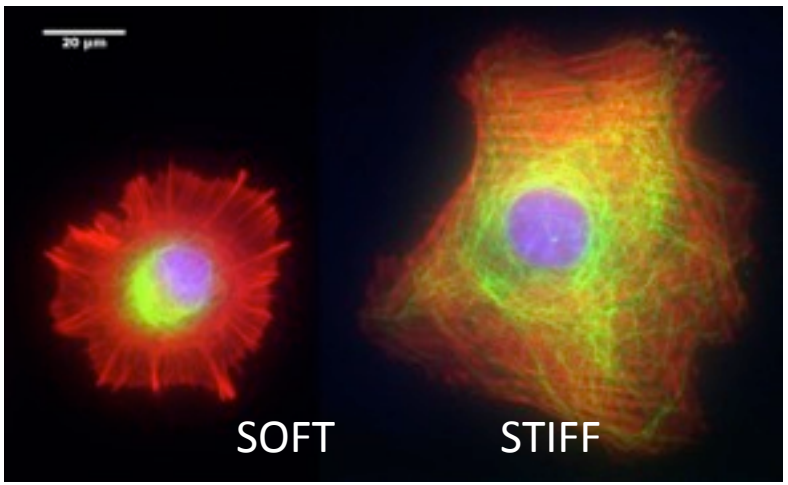
Cell polarization in response of substrate stiffness:

plausible explanation of duratoxis (Lo et al. BJ. 2000)

Non-motile cell on soft substrate: Centripetal movement of actin fibers



REF 52 cell
Actin red
MT green
Nucleus blue



- Confinement of MTs on soft substrates
- MTs are growing up to the cell edge on stiff substrates
- Cell polarity? Durotaxis