Basics about cellular biology

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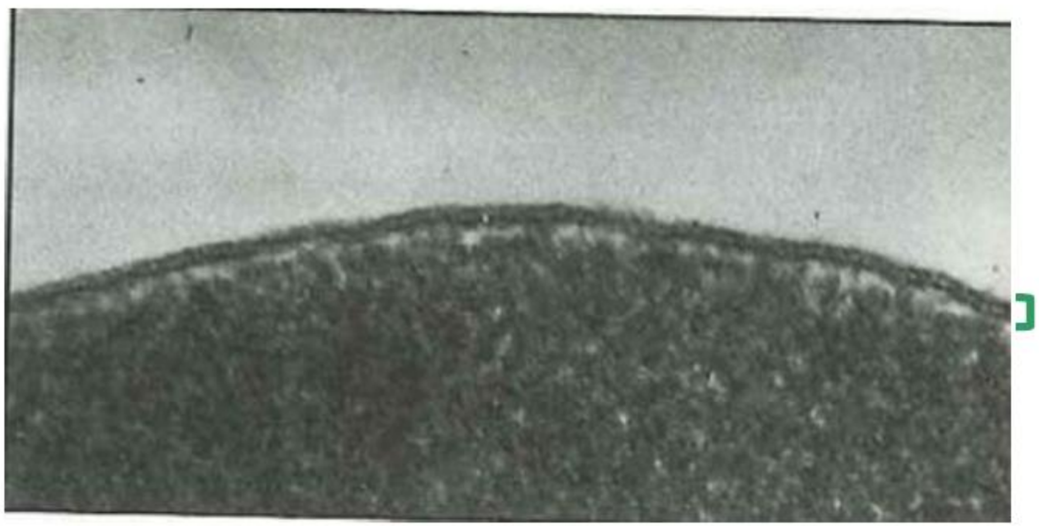
The cell membrane

Envelope that acts as a selective barrier between cytoplasm and extracellular medium.

Without plasmic membrane, the cell could not maintain its ordered chemical system integrity.



Cell membrane

5 or 6 nm thick

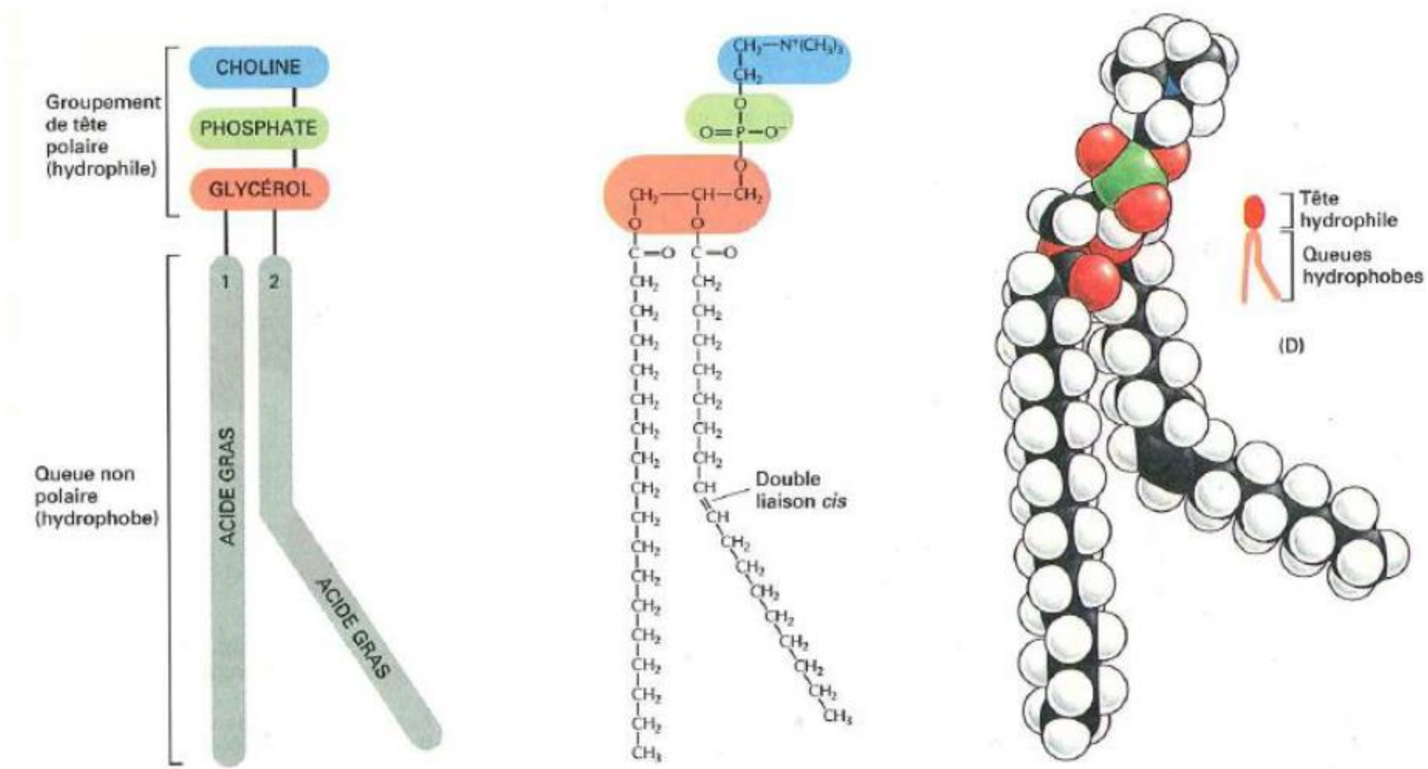
Composition: lipids and proteins

The lipid bilayer

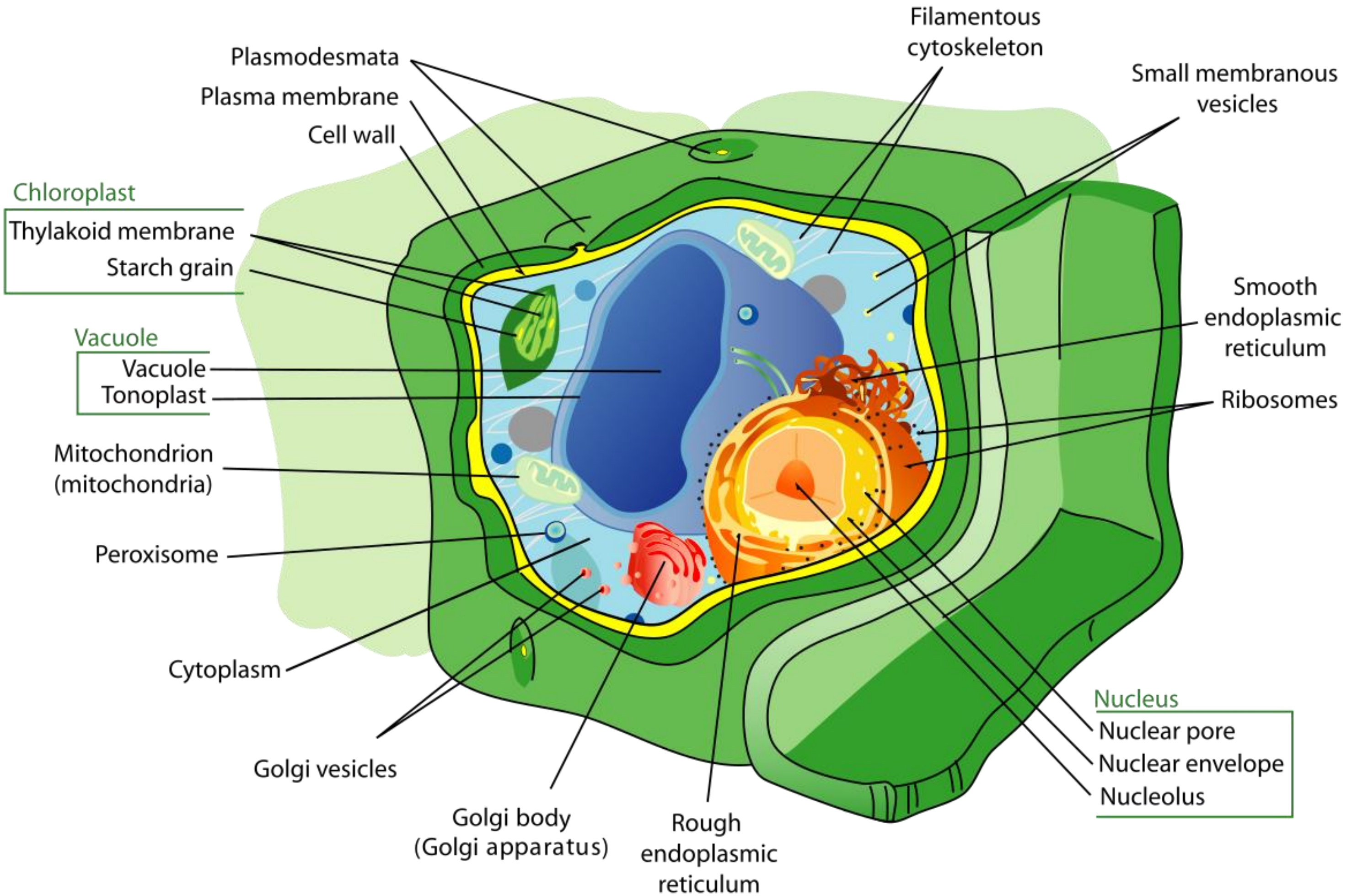
3 main types of membrane lipids:

- Phospholipids (the most numerous)
- Cholesterol
- Glycolipids

Structure of lipids : Polar head — hydrophilic; 2 hydrocarbon tails, Fatty acid — hydrophobic



Structure of plant eukaryote cell



Plastids are found in plants and algae.

The best known are **chloroplasts**, in the cells of photosynthetic organisms, which convert light energy into chemical energy used to make sugars from carbon dioxide.

They also have their own genome.

In plants, algae and fungi, the cell is surrounded by a **pectocellulosic cell wall** which provides the body with a skeleton. Deposits of compounds such as suberin or lignin modulate the physicochemical properties of the wall, making it more solid or more impermeable.

Layout

I. Definition and general presentation of the cell.

II. The main cellular structures.

III. The origin of cells.

IV. The different cellular organizations.

V. Cellular homeostasis.

VI. Structure of the eukaryotic cell:

1. Animal:
    a. The organelles.
    b. The membrane.
2. Plants.

VII. Genetic information.

Genetic information

DNA = Deoxyribonucleic acid

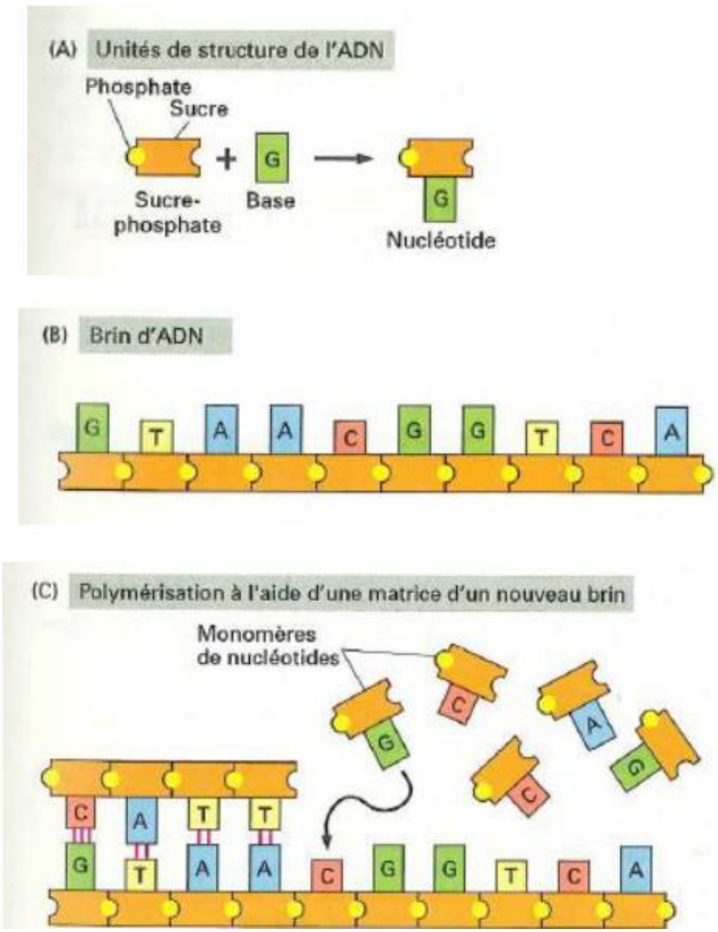
phosphate

Sugar desoxyribose

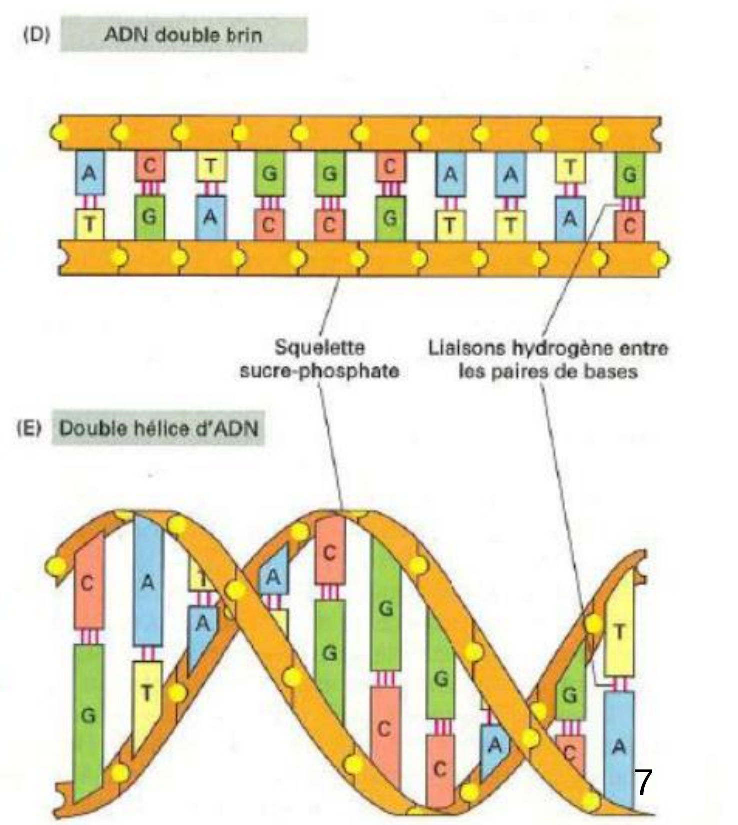
base

A= Adénine
T= Thymine
C= Cytosine
G= Guanine

Unité de base → Brin d'ADN → Double brin d'ADN

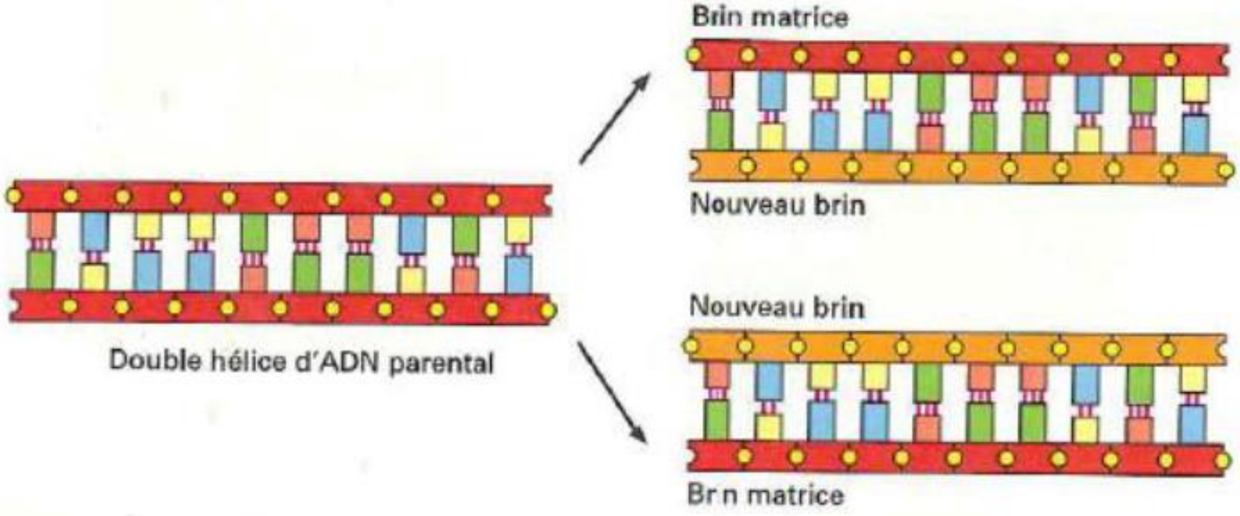


Double brin d'ADN → Double hélice d'ADN



- Duplication of genetic information by reppling DNA:

➔ polymerization using a matrix.

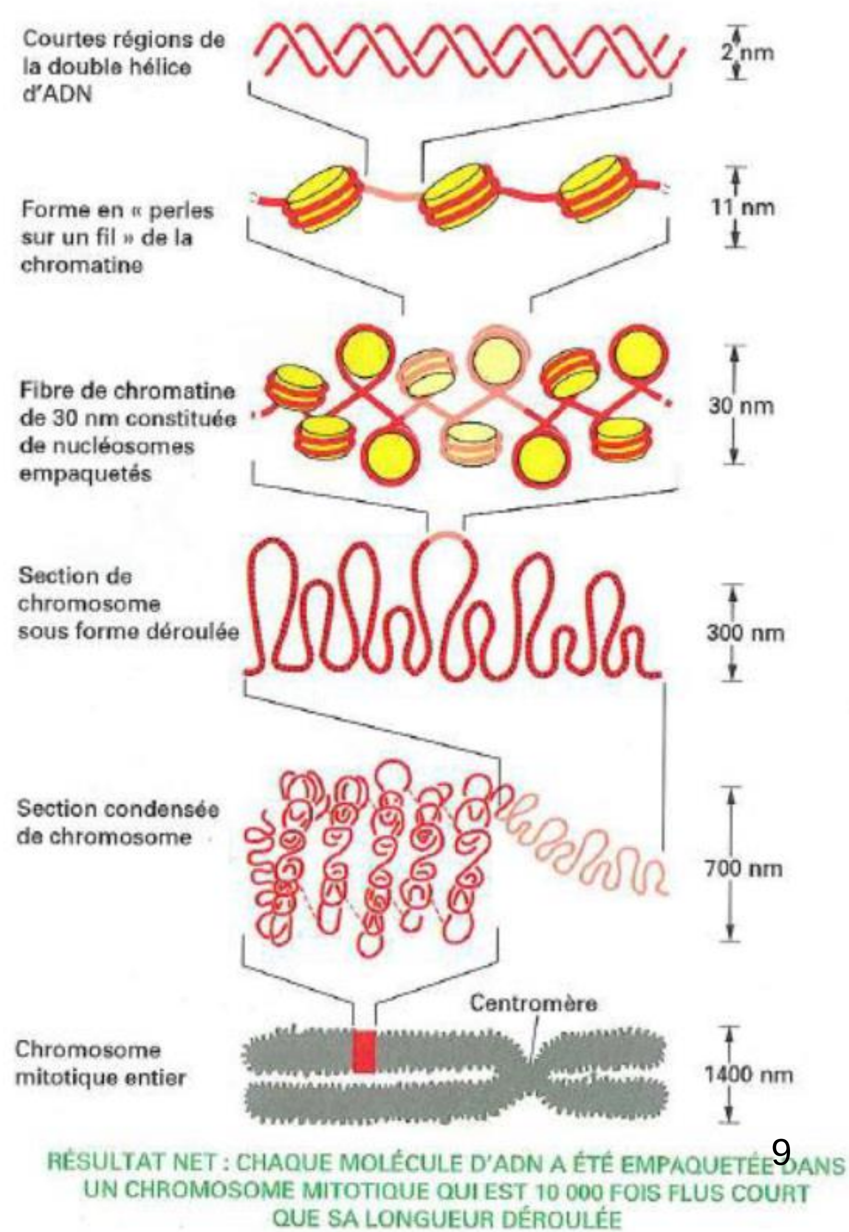


Each cell contains 2 m of DNA. The nucleus measures 6 µm diameter
➔ compaction of the DNA with proteins

23 pairs of chromosomes

22 pairs of homologous chromosomes

1 pair of sexual chromosome XX or XY



Cellular mechanics

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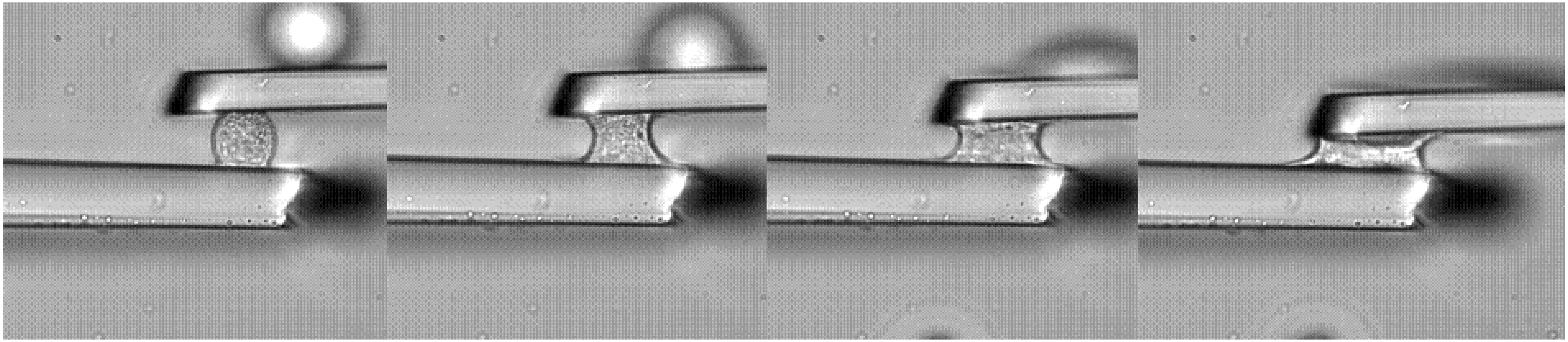
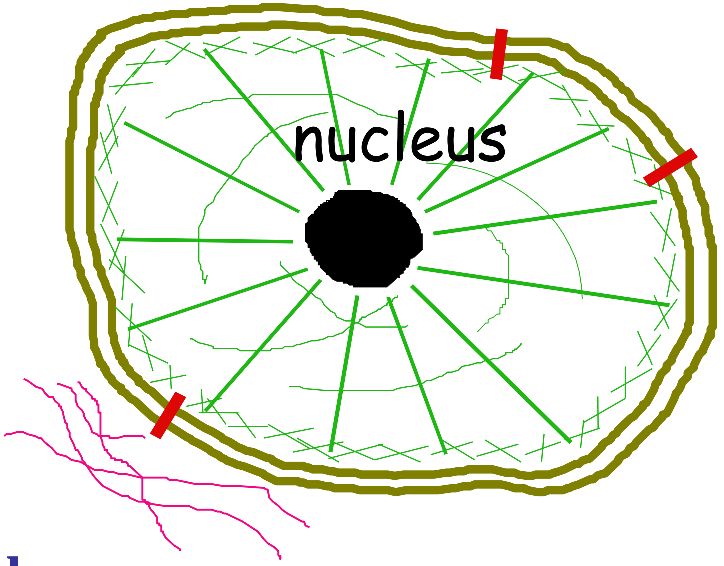
Passive and active mechanical properties of isolated living cells



Cell and mechanics



Passive properties

Viscoelasticity of tissues

structure ↔ propagation of forces

Active properties

Mechanical response to a biochemical signal

chemo-attractant, adhesion → polarisation, migration

Mechanical / biochemical response to a mechanical signal :

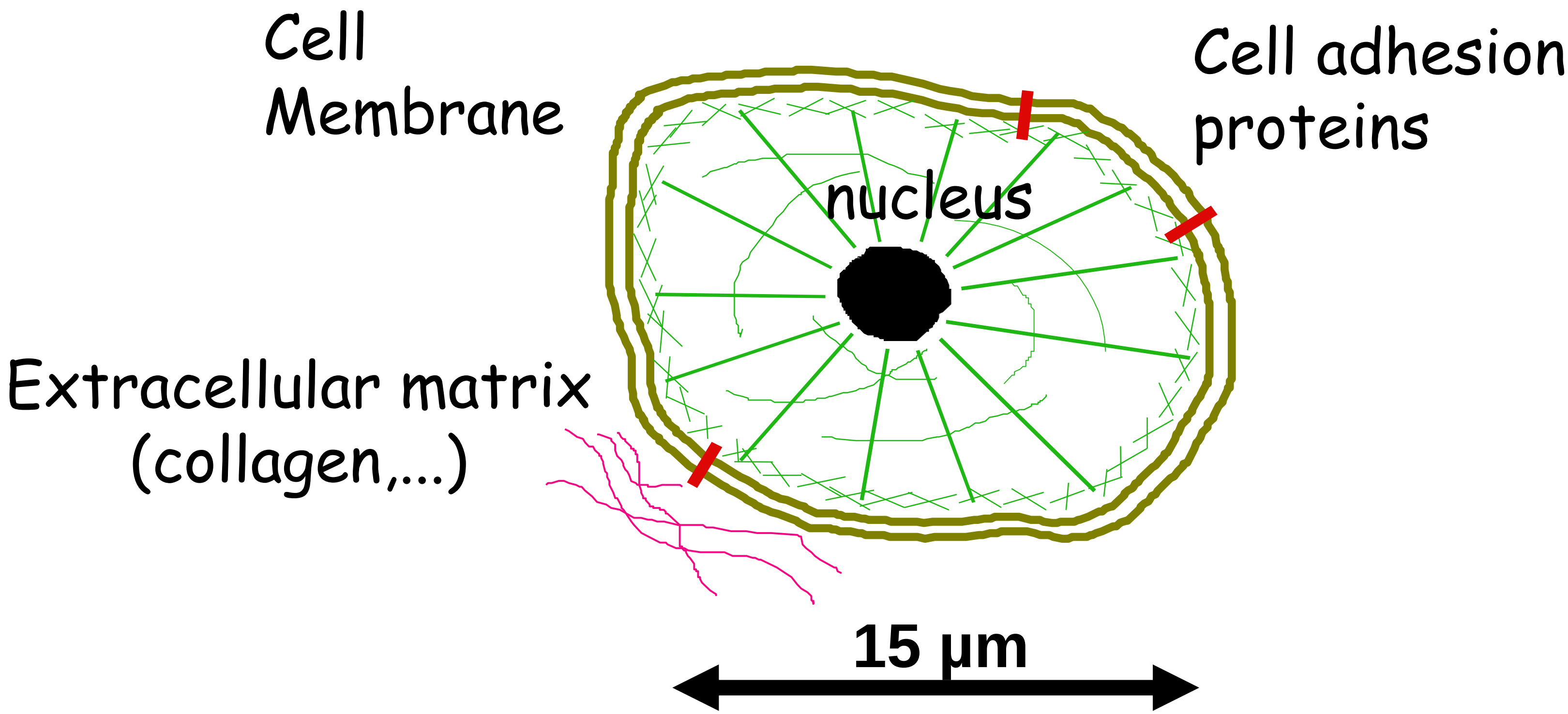
mechanotransduction

cultured cells: always under tension

secretions of an epithelium modulated by blood flow

Mechanics ↔ Biological function

Cell and mechanics

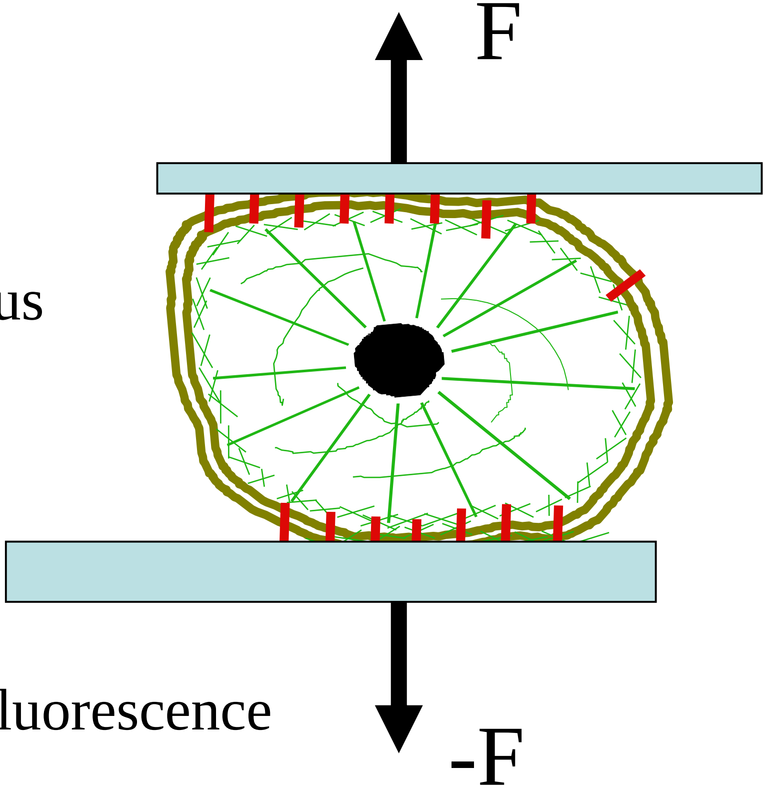


Dynamic cytoskeleton (2D-3D) :

- Actin filaments (Ø=8nm, Lp=15µm)
- Intermediate filaments (Ø≈10nm, Lp≈500nm)
- Microtubules (Ø=25nm, Lp ≈ qq mm)

Passive properties

Apply a **controlled stress** to the cell and determine its :



Mechanical response

Strain measurement (creep)

Assessment of the viscoelastic modulus

With collaborations

Structural response

visualization of the cytoskeleton by fluorescence

Biochemical response (genetics)

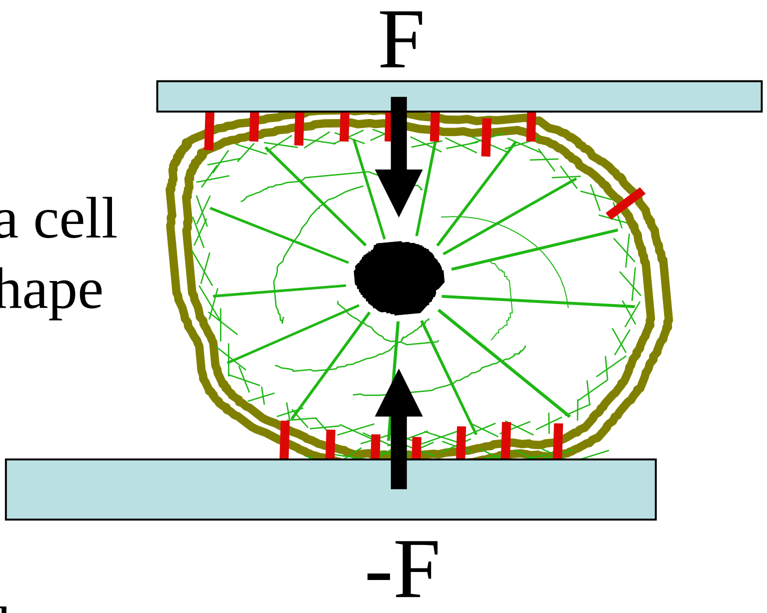
monitoring of protein or genetic markers

Active properties

Observe the evolution of a cell in a predefined and simple geometry (3D):

Mechanical activity

Measurement of the force applied by a cell
Correlation with the evolution of its shape



With collaborations

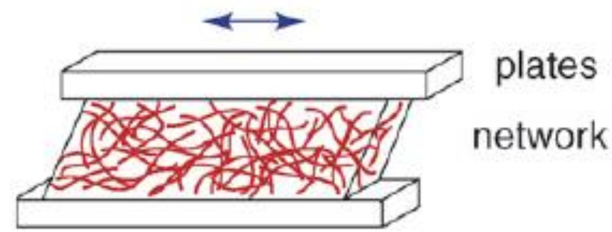
Structural response

visualization of the cytoskeleton by fluorescence

Characterization methods

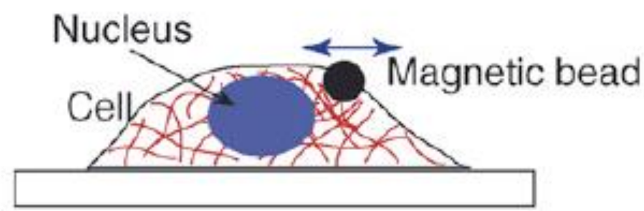
Bulk rheology

A material is sheared between two plates using an oscillatory stress to probe the shear elastic, G', (in-phase) and viscous, G'', (out-of-phase) moduli.



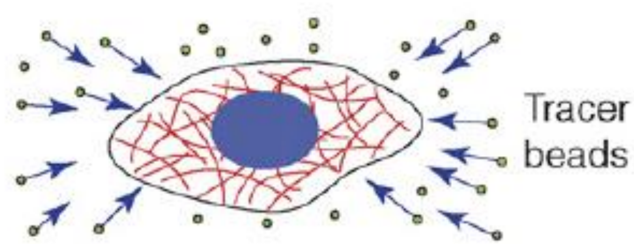
Magnetic bead cytometry

An external magnetic field applies a stress to a magnetic bead. The bead is position tracked to determine the response.



Traction force microscopy

Cell contractions deform a flexible substrate. Forces are estimated from bead displacements.

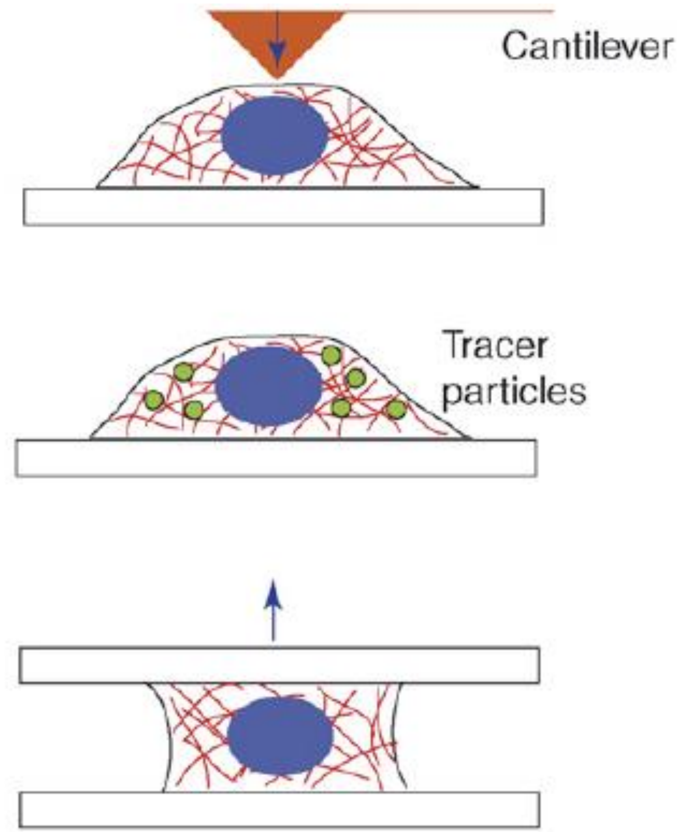


Characterization methods

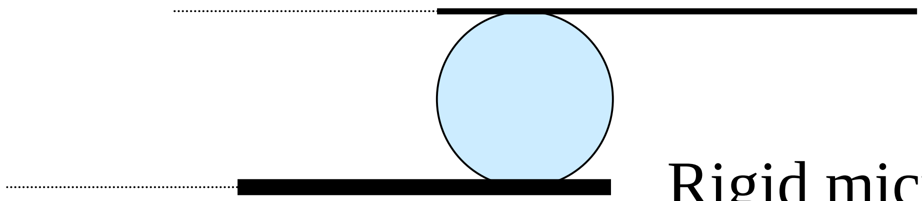
Atomic force microscopy
A cantilever applies stress to the cell. The cantilever deflection is measured by laser reflection.

Microrheology
The motion of probe particles is measured using either video or laser tracking techniques. Particle motion is either driven externally or thermally induced and is interpreted to yield the viscoelastic modulus.

Whole cell stretching
A cell is attached to two surfaces. A force is applied to one surface and the plate separation is measured.

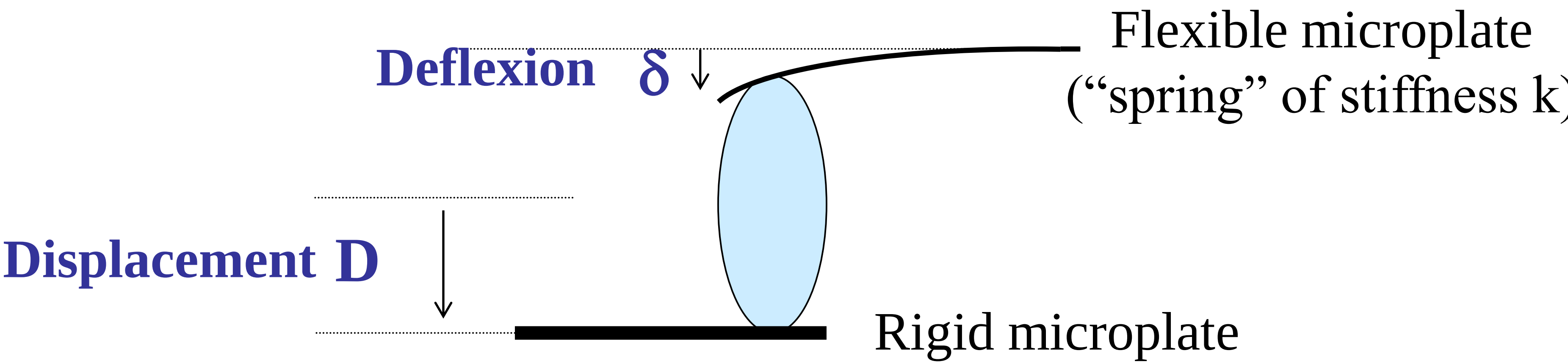


Uniaxial stretching



Thoumine et Ott, *J. Cell Sci.* **110** p 2109 (1997)

Uniaxial stretching



Force $F = k\,\delta$

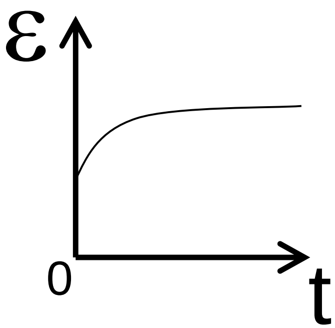
Thoumine et Ott, J. Cell Sci. ***110*** *p 2109 (1997)*

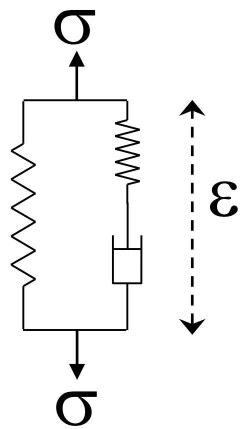
Passive properties:

Rheology of a living cell usig unaxial stretching

Local measurements, time domain

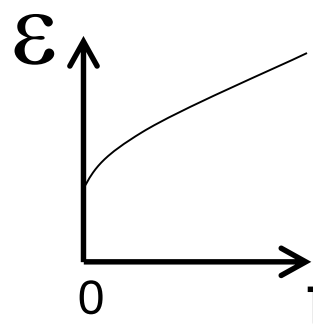
Wu et al., *Scanning*. 2003

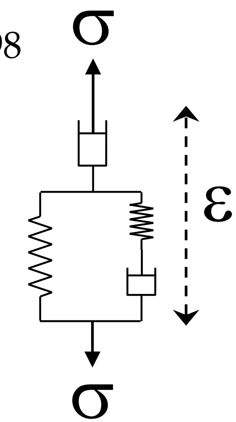




Standard linear solid

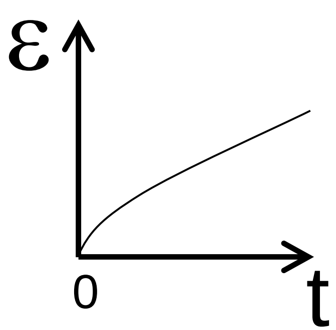
Bausch et al., *Biophys J*. 1998

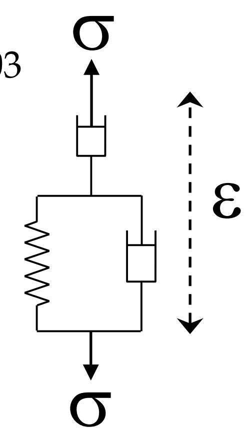




Liquid with instantaneous elasticity

Wilhelm et al., *Phys Rev E*. 2003

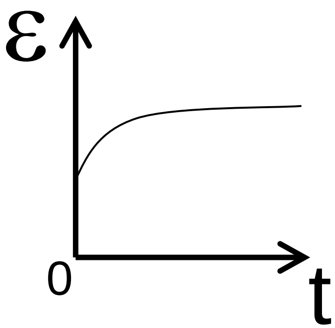


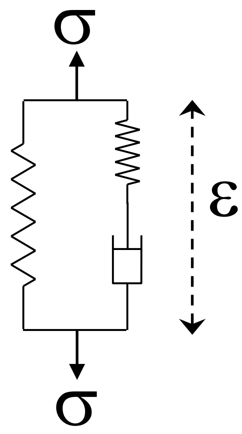


liquid

Local measurements, time domain

Wu et al., *Scanning*. 2003





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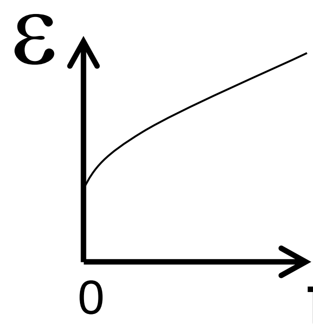

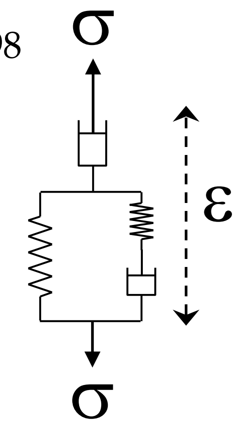

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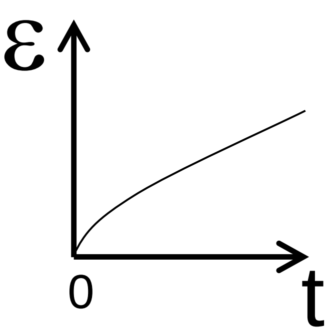
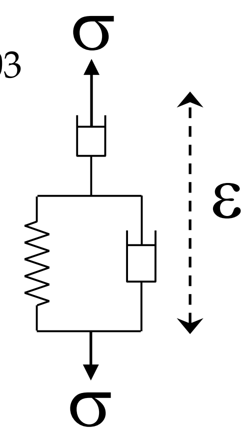




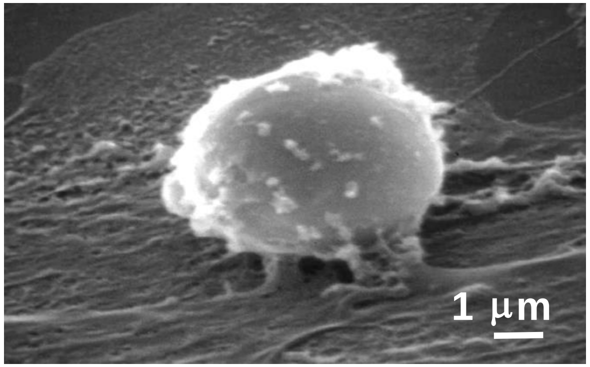
liquid

Some characteristic times

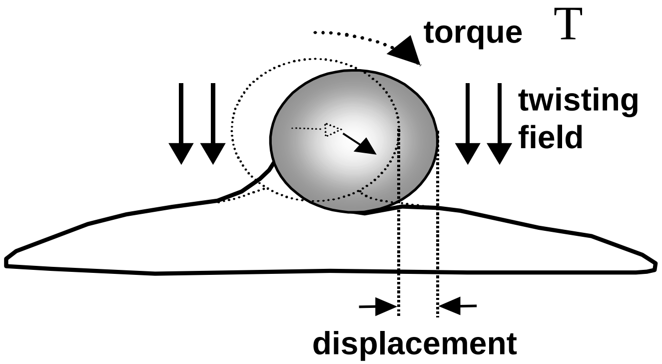
Viscous dissipation

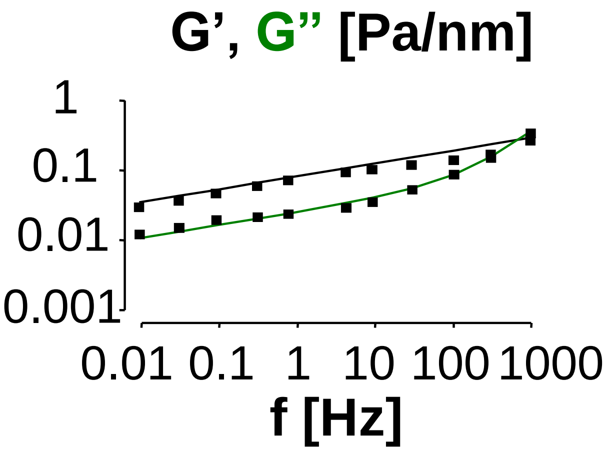
Very different behaviors

Local rheometry, frequency analysis

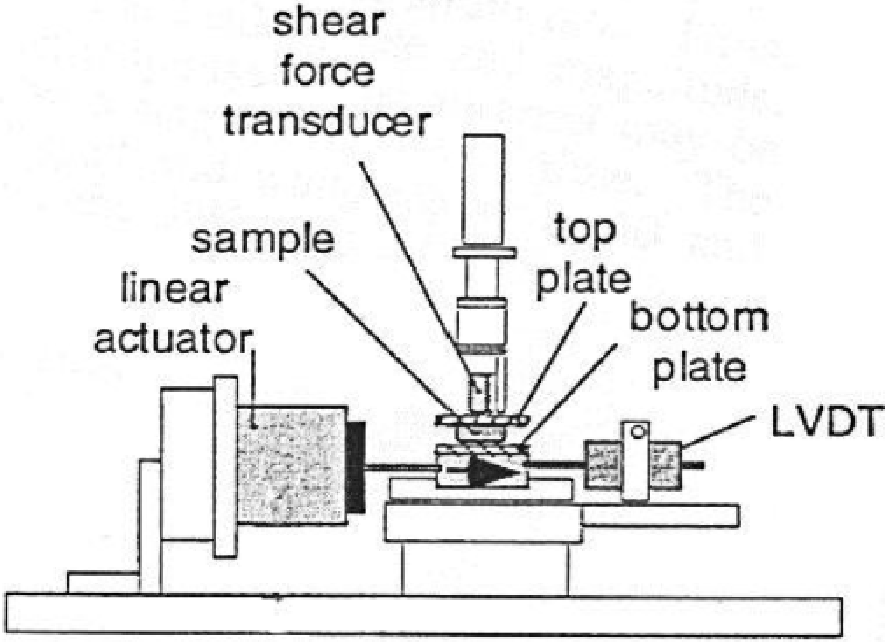


Fabry et al., *Phys Rev Lett.* 2001

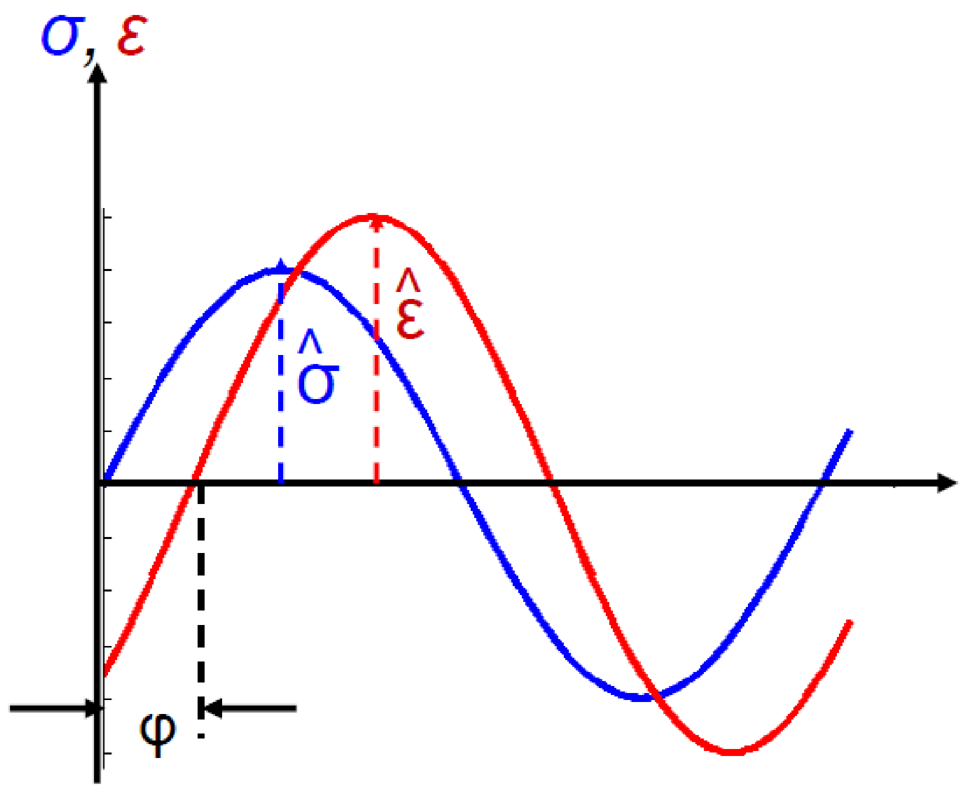




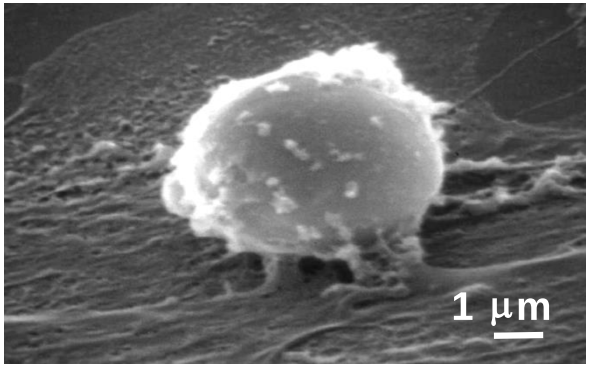
Oscillations to determine viscoelastic properties



$$\varepsilon = \hat{\varepsilon}\sin(\omega t)$$



Local rheometry, frequency analysis



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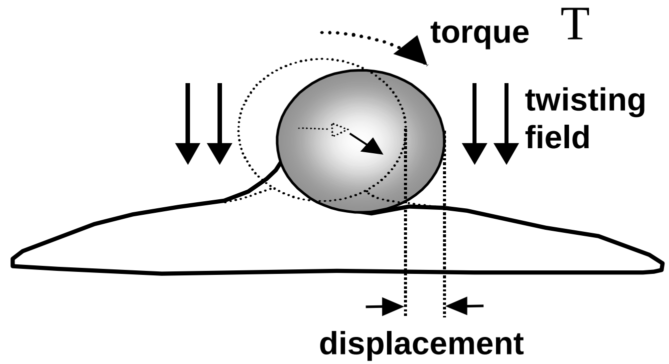
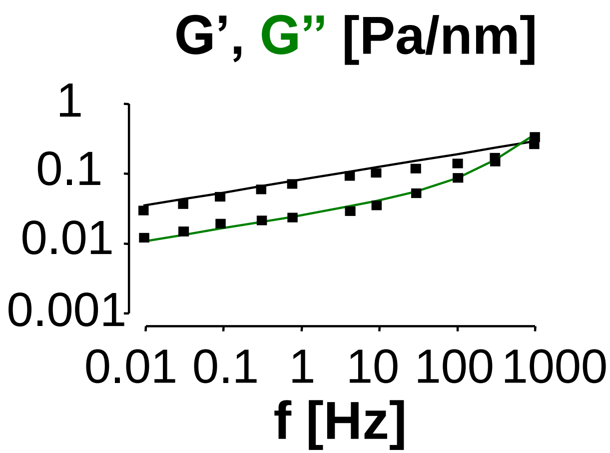


No characteristic time

Elasticity and dissipation from same origin

Unique behavior preserved



AVERAGE !

$$G^*(\omega) = G_0\Gamma(2-x)(j\,\omega\tau_0)^{x-1} + j\omega\mu$$

$$\Gamma : z \mapsto \int_0^{+\infty} t^{z-1}\, e^{-t}\, \mathrm{d}t \qquad \Gamma(z+1) = z\,\Gamma(z).$$

Soft glassy medium behavior

- Out of balance
- Structural disorder
- Metastability
- Effective temperature (glass transition)

What if the curve of the model does not fit the curve of the material we want to describe?

Generalized Models…

- The strain response to an arbitrary stress history is obtained from J(t) by *superposition*

$$\varepsilon(t) = \int_0^t J(t-\tau)\,d\sigma = \int_0^t J(t-\tau)\frac{d\sigma}{d\tau}d\tau$$

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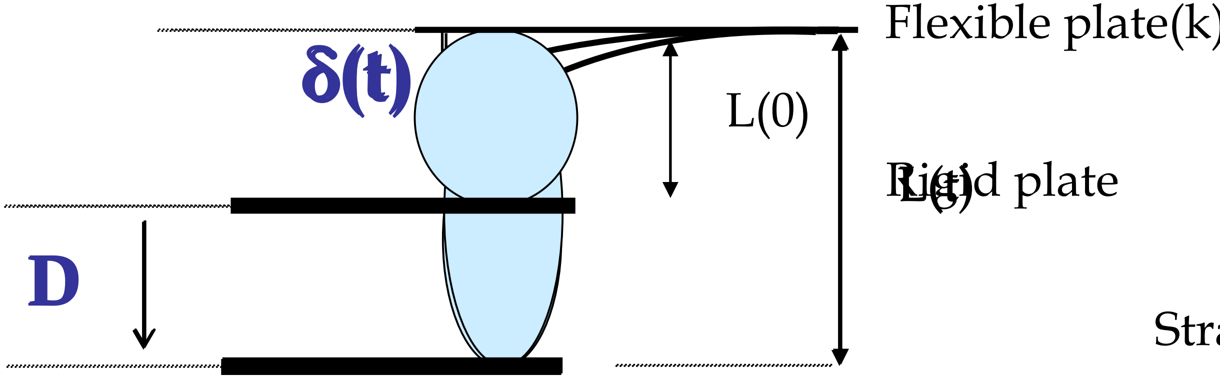
1. From uniaxial stretching to single cell rheometer

Uniaxial stretching $(\dot{\sigma} \neq 0 \text{ et } \dot{\varepsilon} \neq 0)$



Stress :

$$\sigma(t) = \frac{F(t)}{S}$$

$$F(t) = k.\delta(t)$$

Strain :

$$\varepsilon(t) = \frac{\mathrm{L(t)} - \mathrm{L(0)}}{\mathrm{L(0)}}$$

Stress-strain relationship

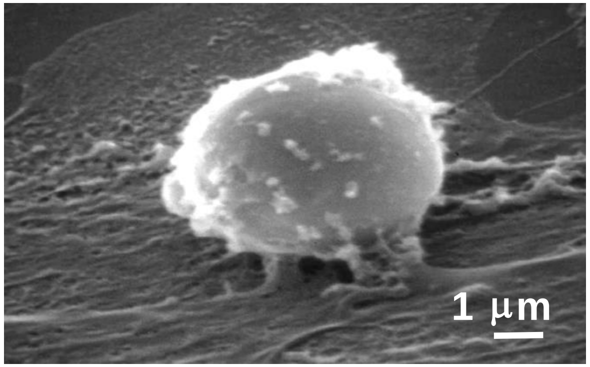
$$\varepsilon(t) = J(t)\sigma(0) + \int_0^{+\infty} J(t-t')\dot{\sigma}(t')dt'$$

$\dot{\sigma} \neq 0 \Rightarrow$ Very difficult to determine J

Avoid convolution product ⇔ oscillations (σ(ω))

ou constant stress $(\dot{\sigma} = 0)$

Local rheometry, frequency analysis



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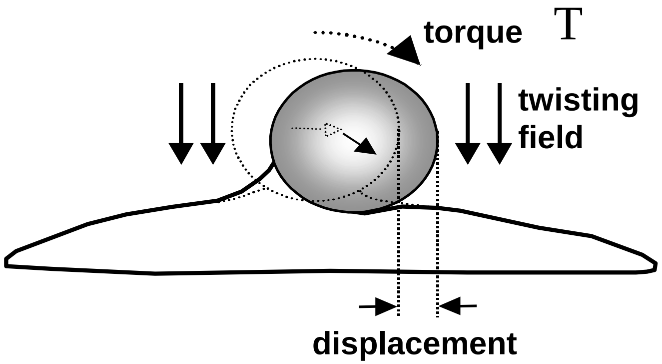

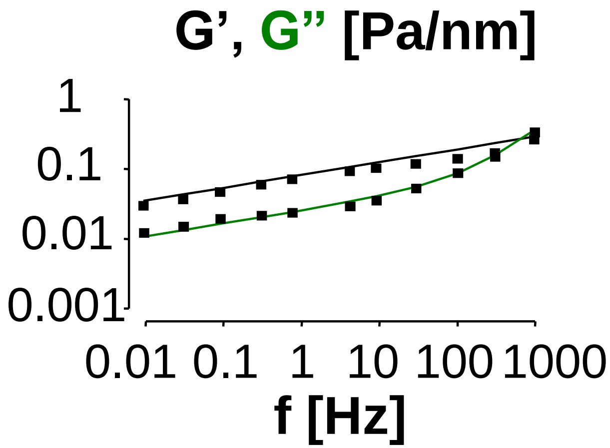

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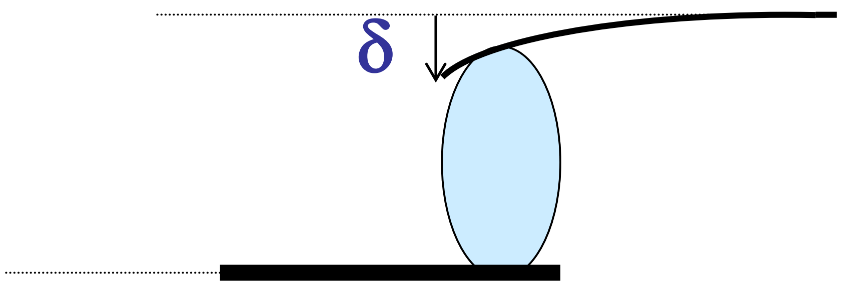
1. From uniaxial stretching to single cell rheometer

Rheometer $(\dot{\sigma} = 0)$

$$\varepsilon(t) = J(t)\sigma(0)$$

At constant stress: measurement of J ⇔ mesurement of strain ε



F = k.δ

$\dot{\sigma} = 0$ ⇔ δ **constant**

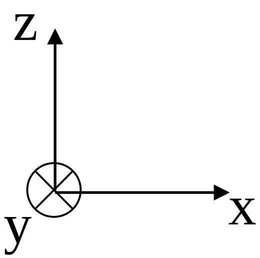
$$\delta = 10\mu m$$

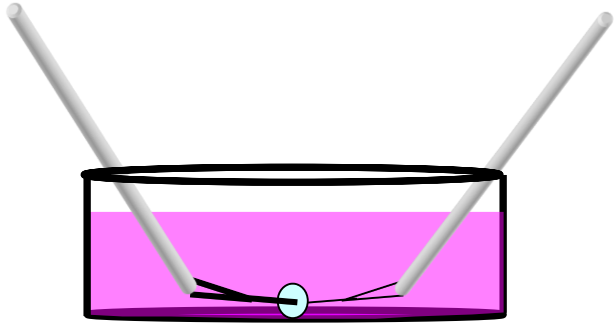
$$O,O3nN.\mu m^{-1} < k < 15nN.\mu m^{-1}$$

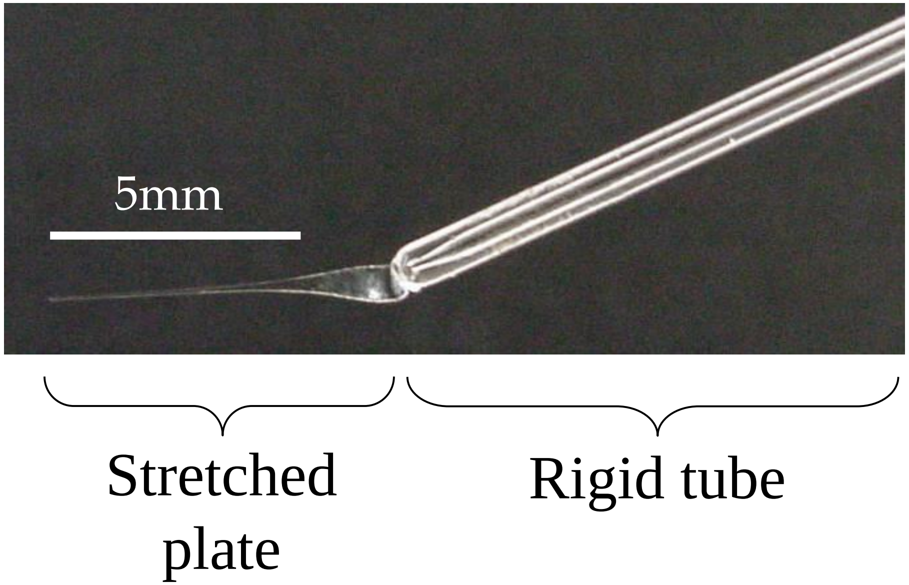
$$300\,pN < F < 1\mu N$$

Single cell rheometer

Desprat et al., Rev.Sci. Instrum. ***77****, 055111-1 (2006)*







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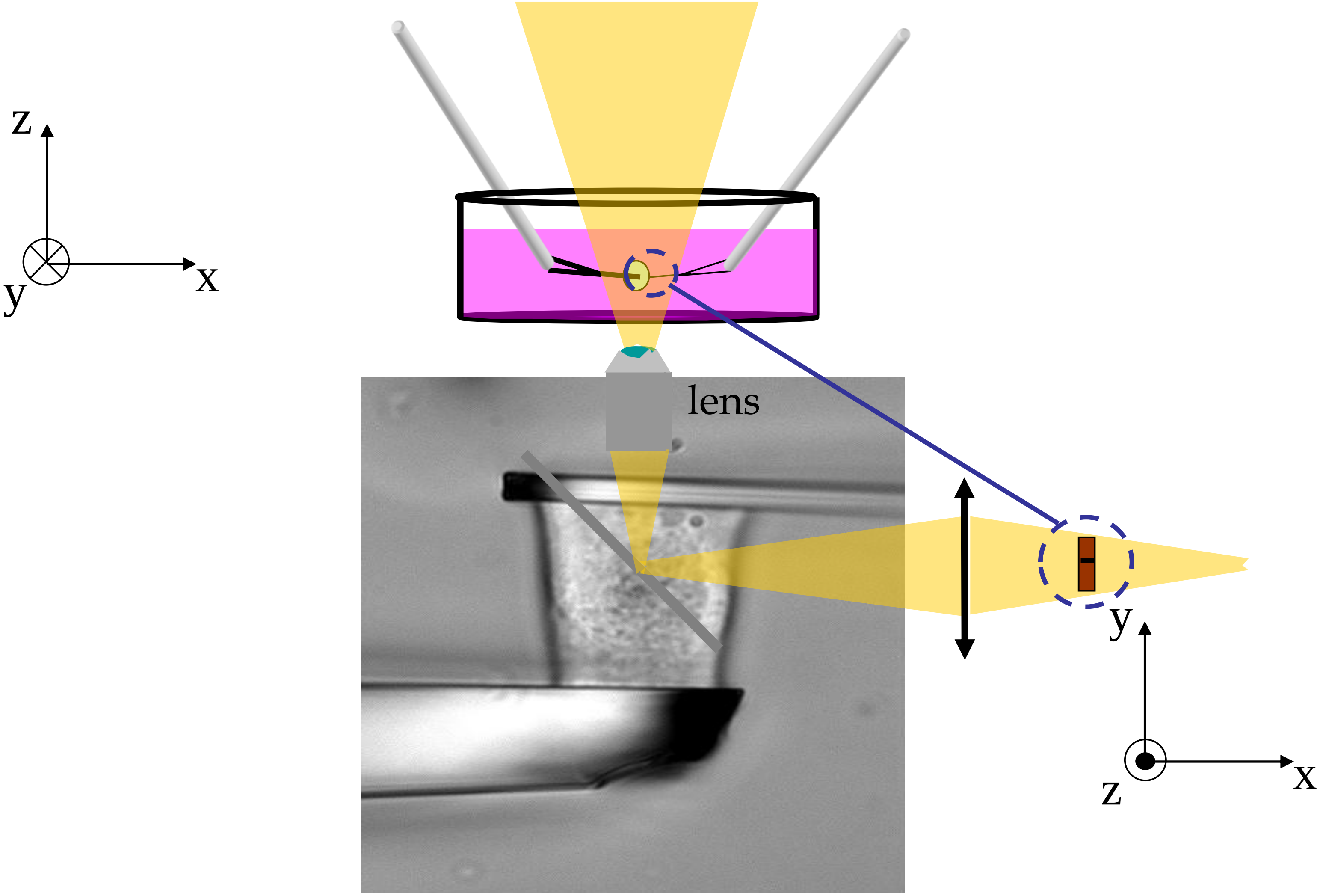
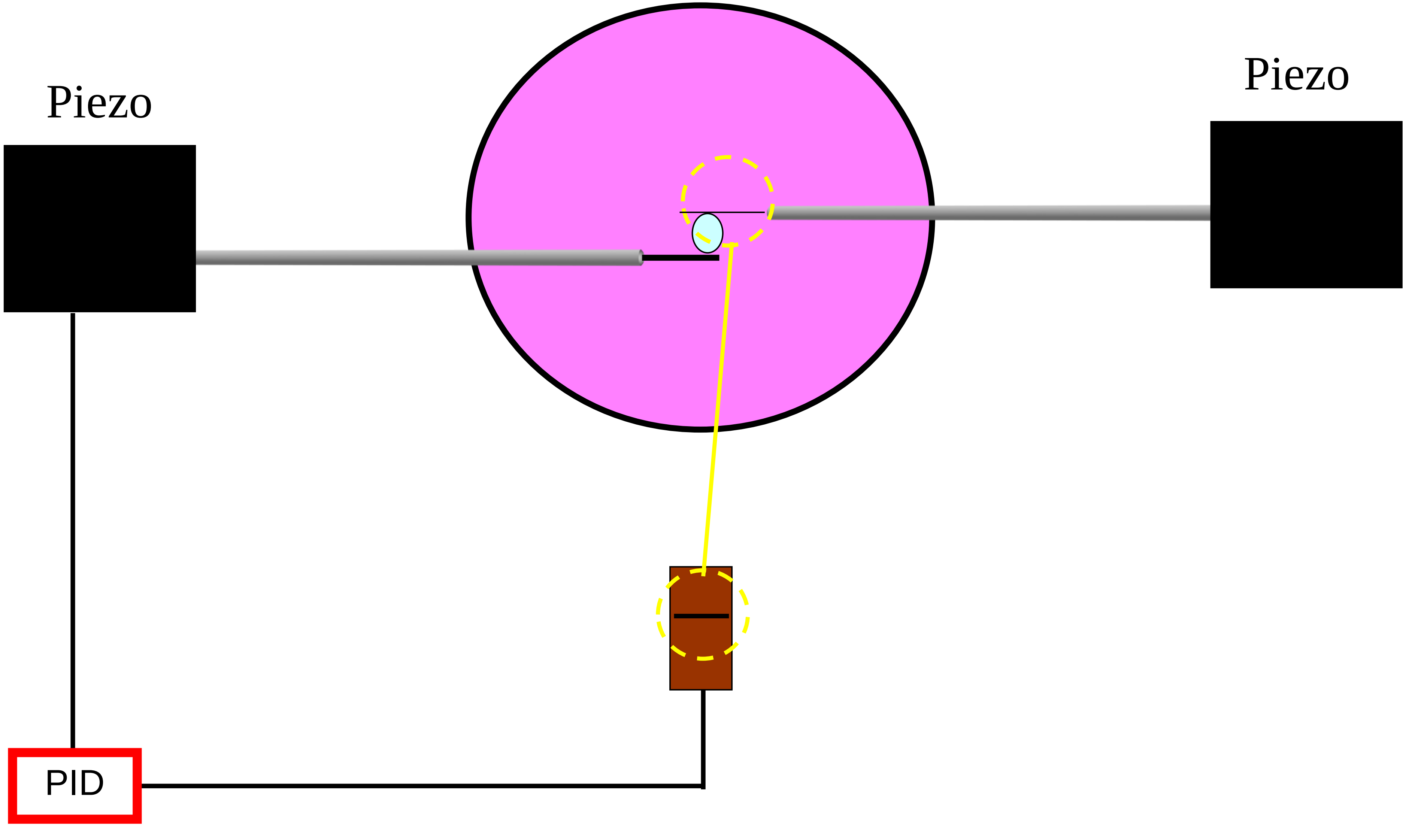


Creep experiment



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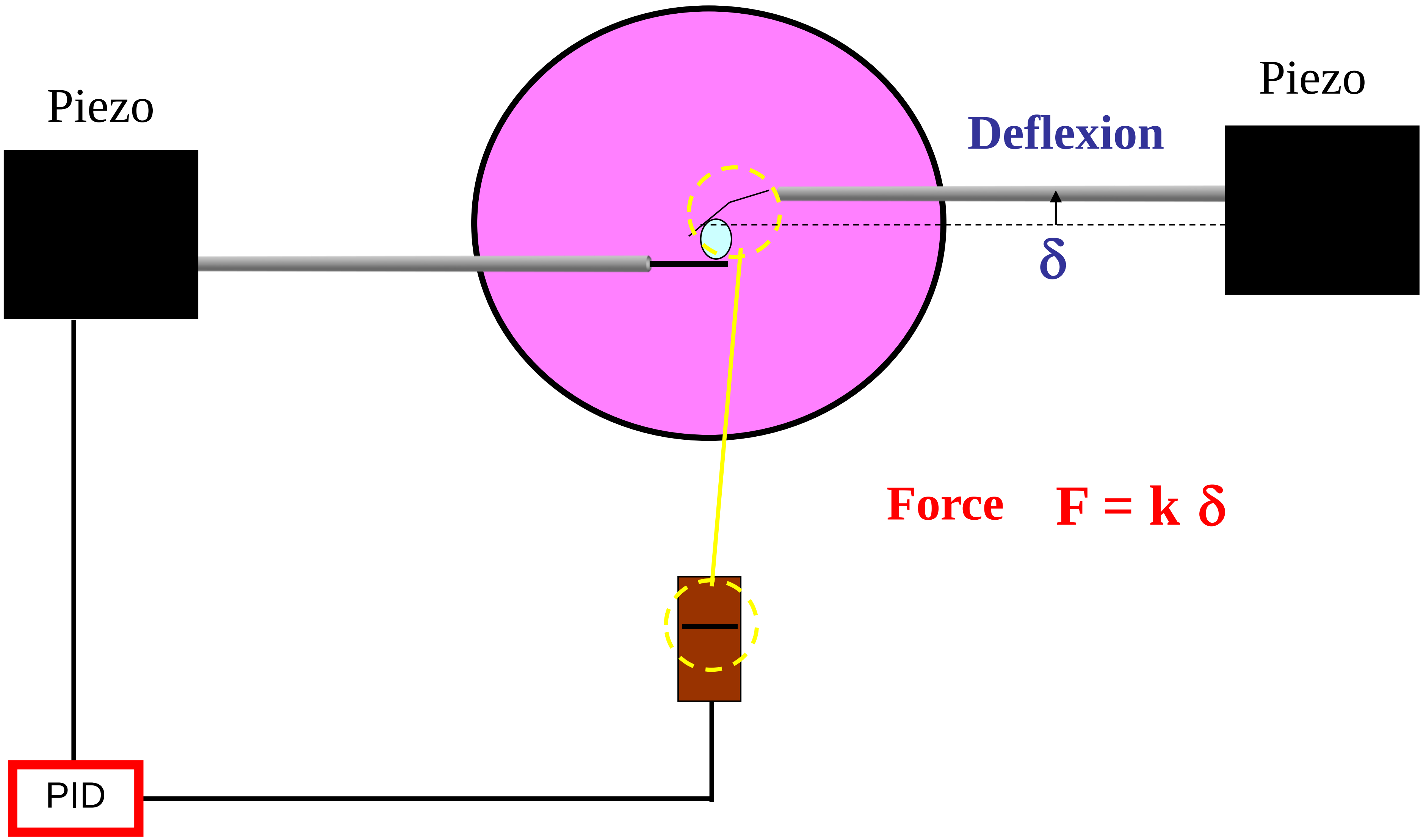

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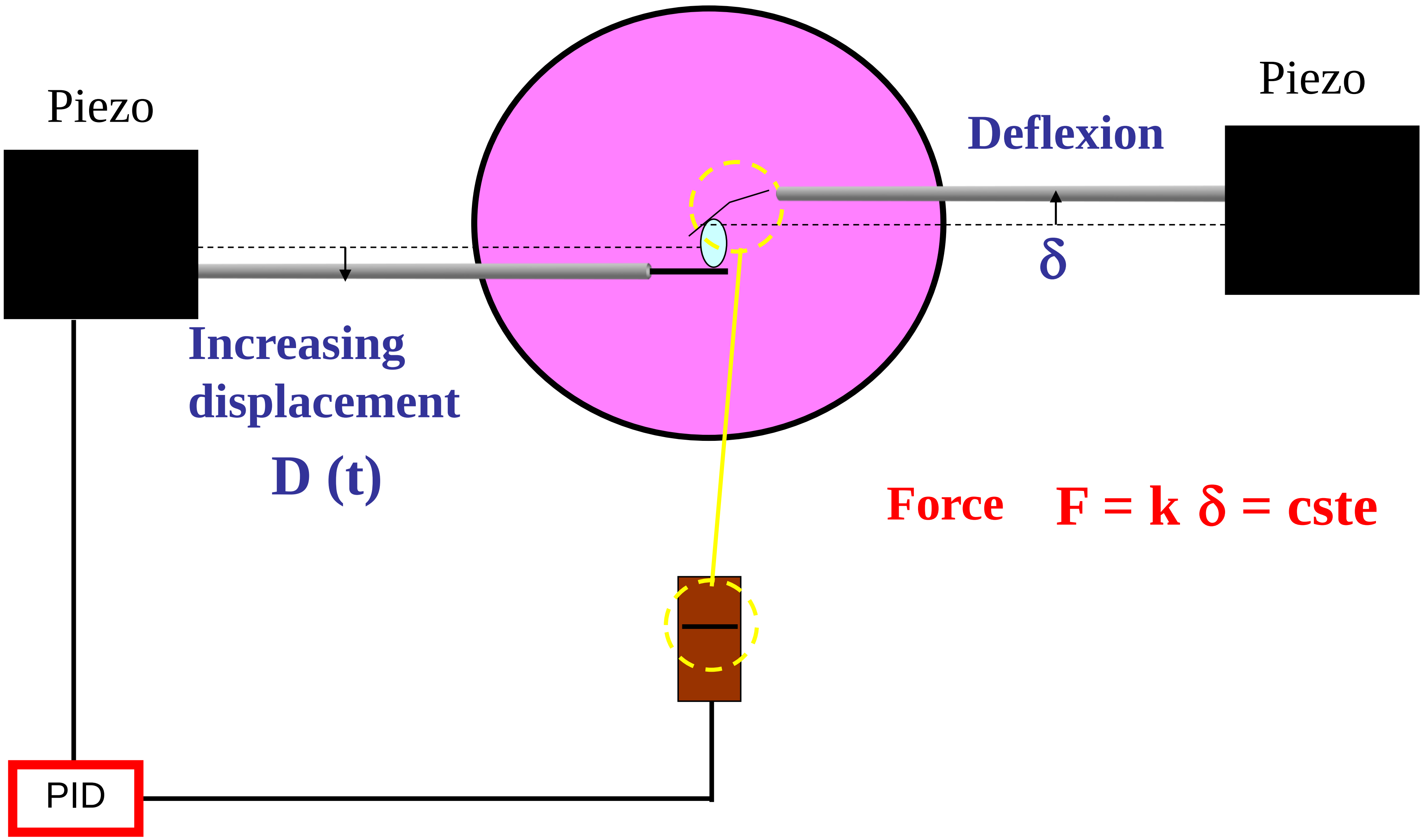

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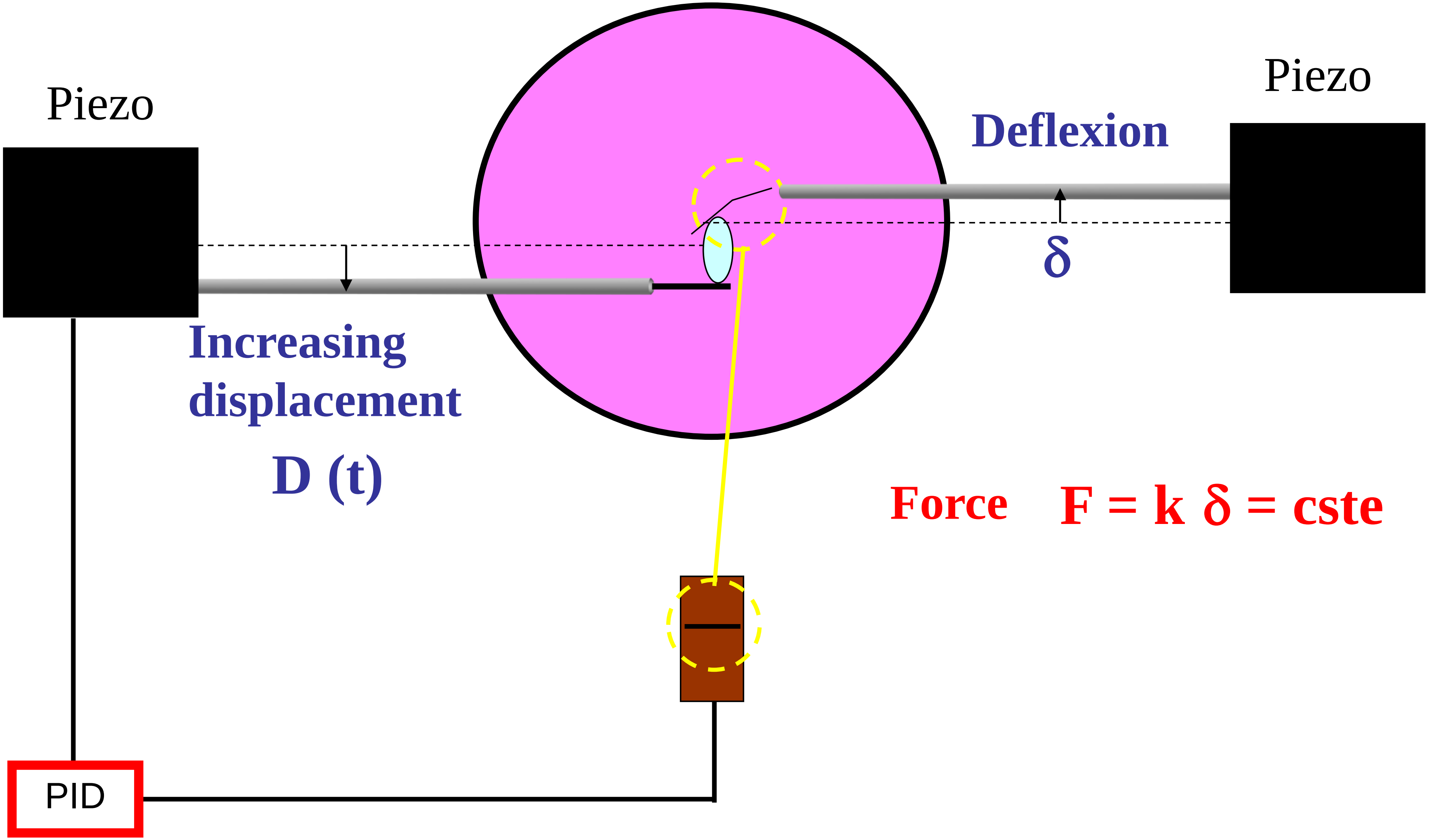
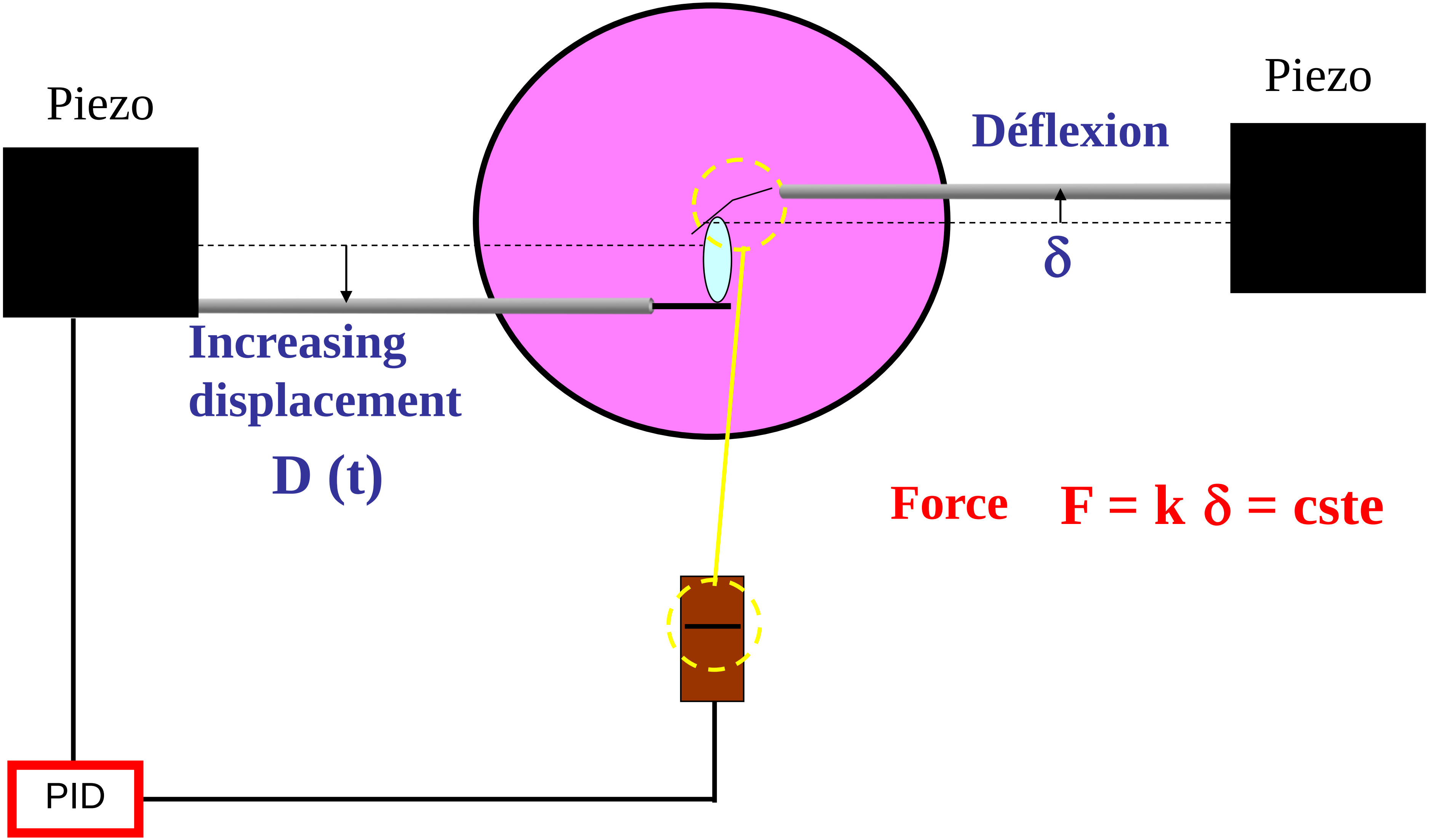
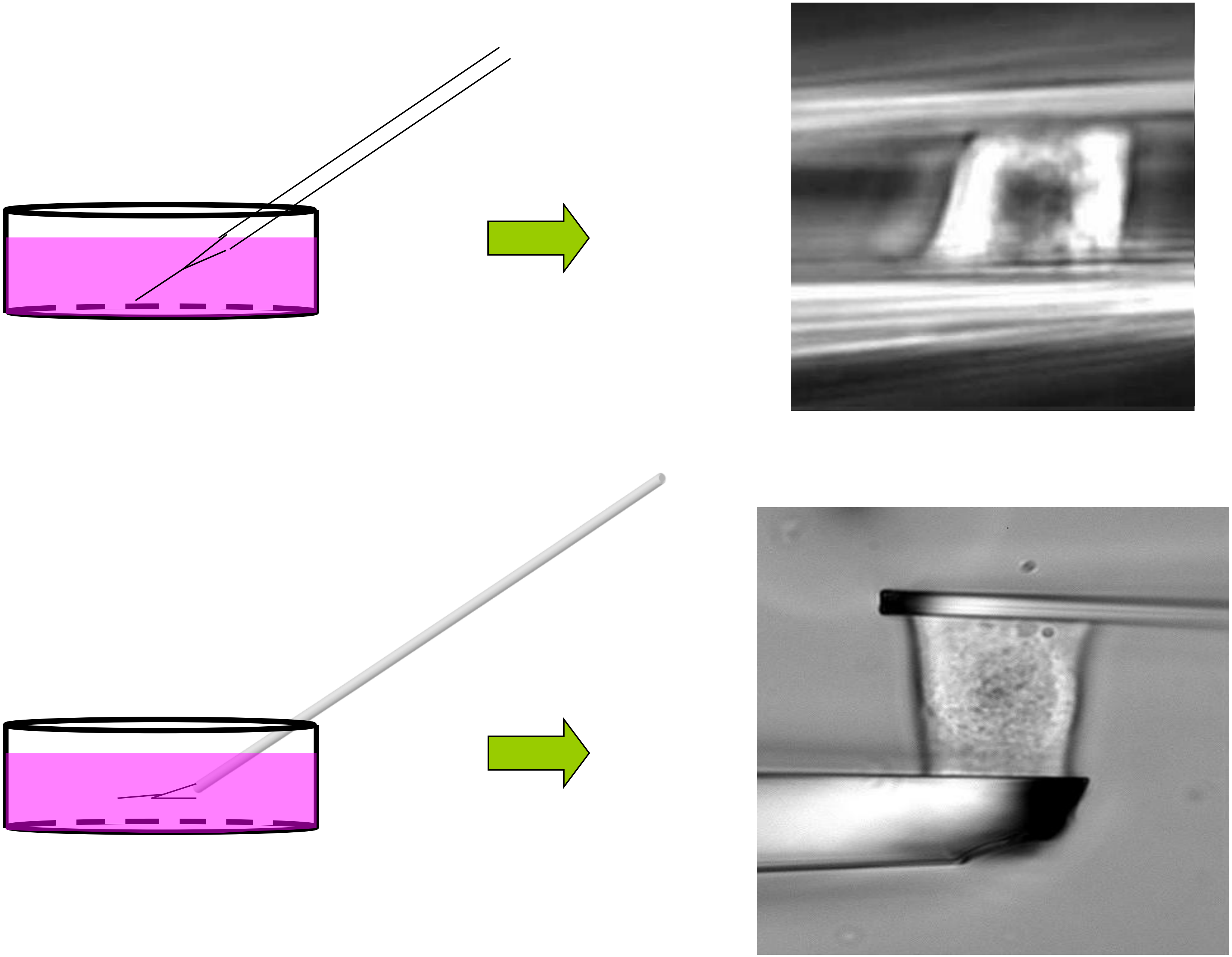


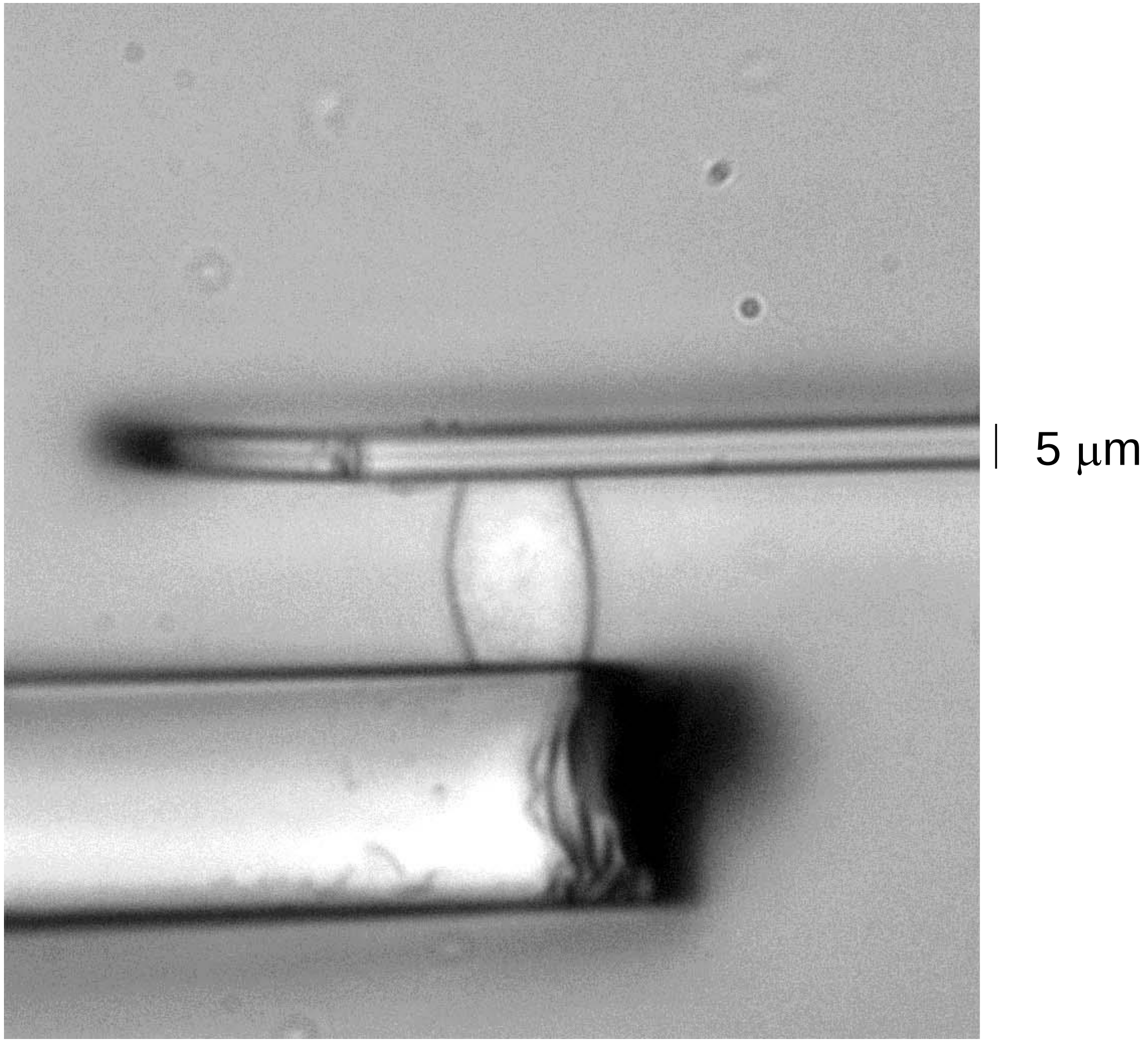
Creep experiment



Cell stretching D(t) at constant force

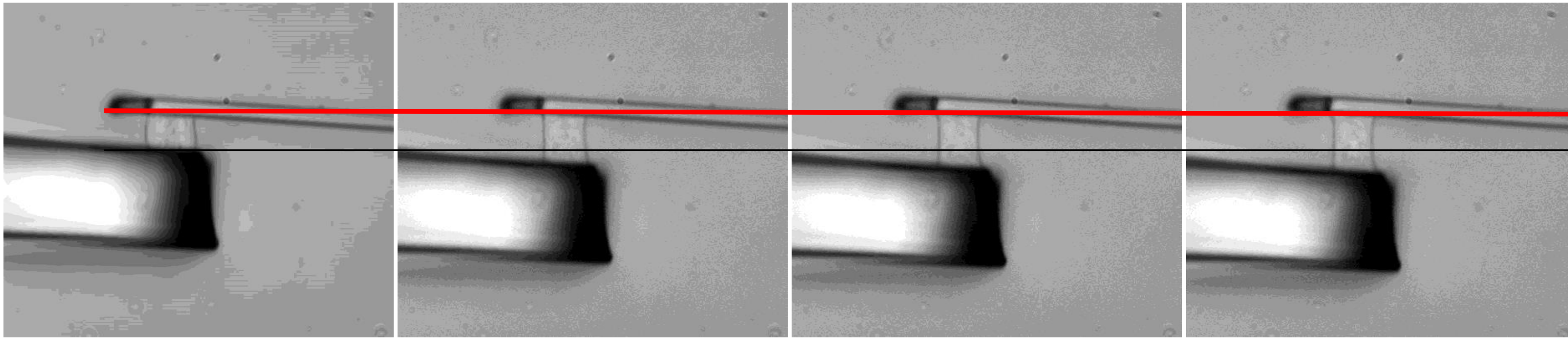


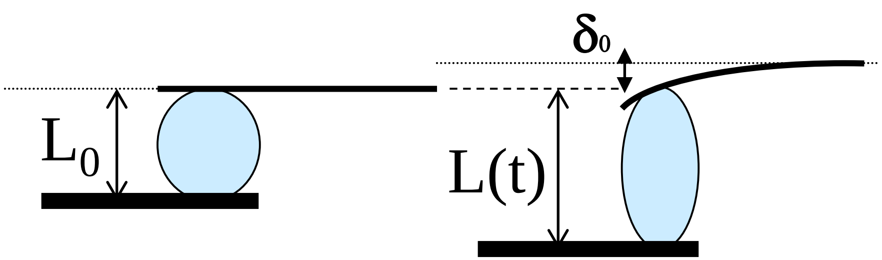
Creep experiment

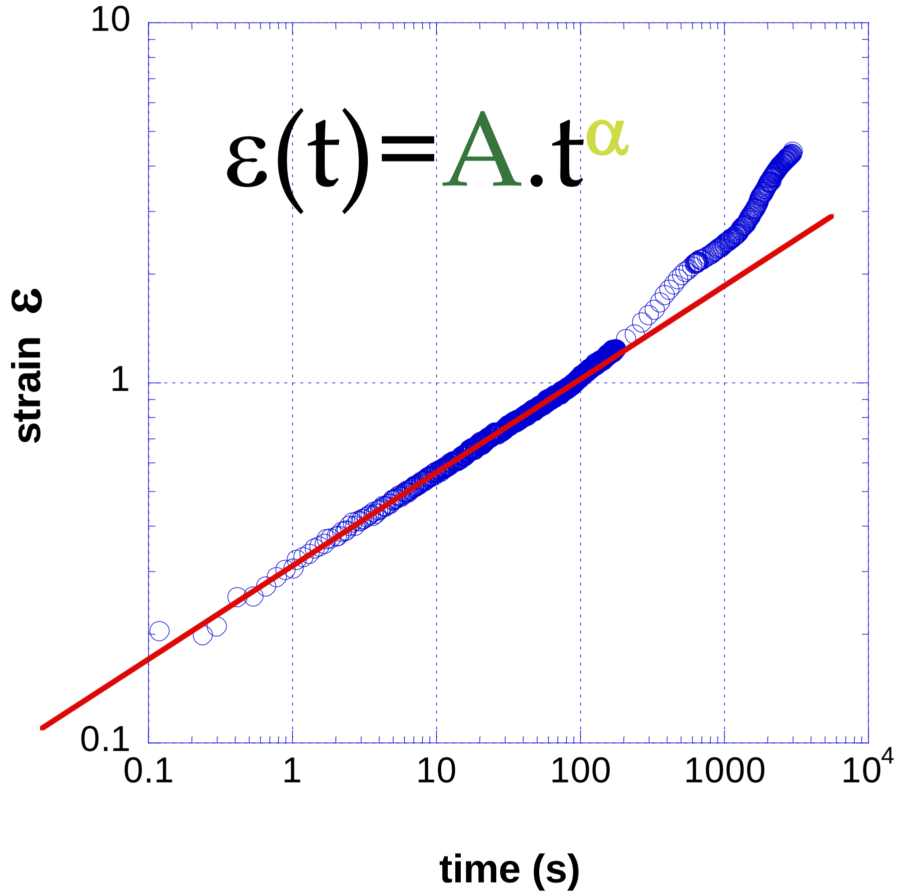


Plates treated with Glutaraldehyde, non specific adhesion

Creep experiment







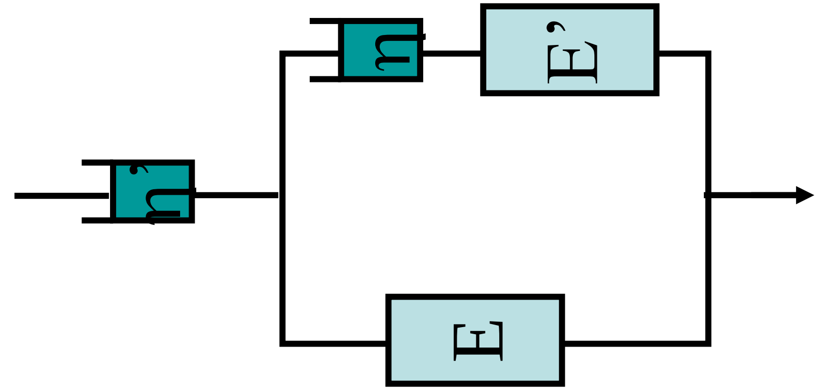
Force $F_0 = k\ \delta_0 = cst$

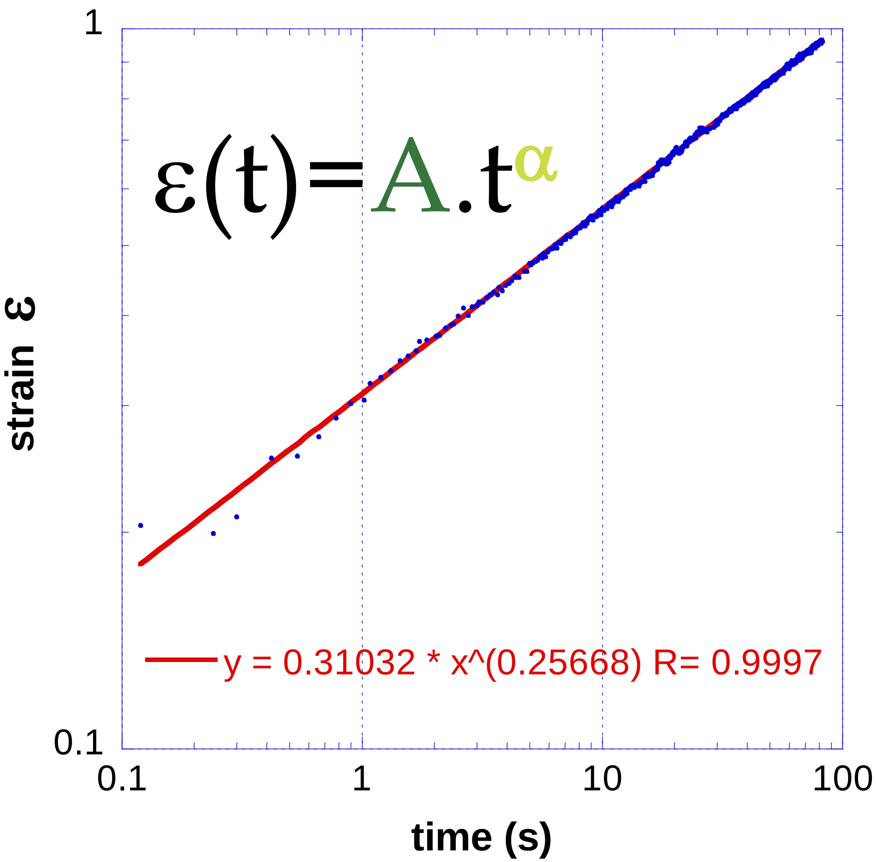
Strain $\varepsilon(t) = \dfrac{L(t) - L_0}{L_0}$

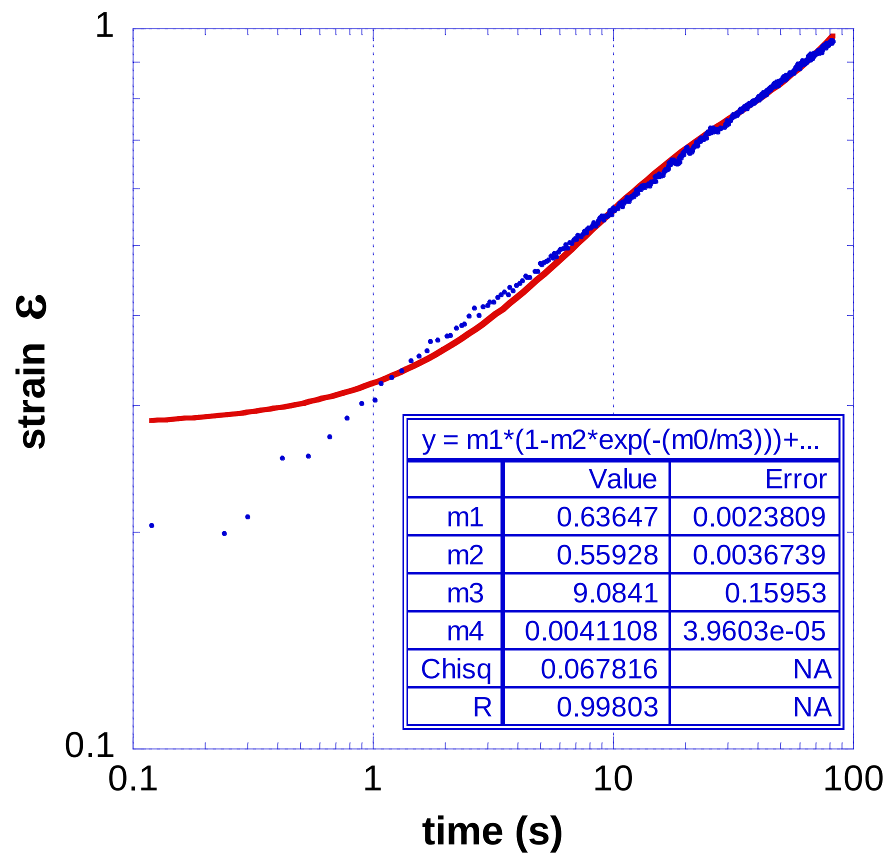
No characteristic time

Comparison with simple mechanical models

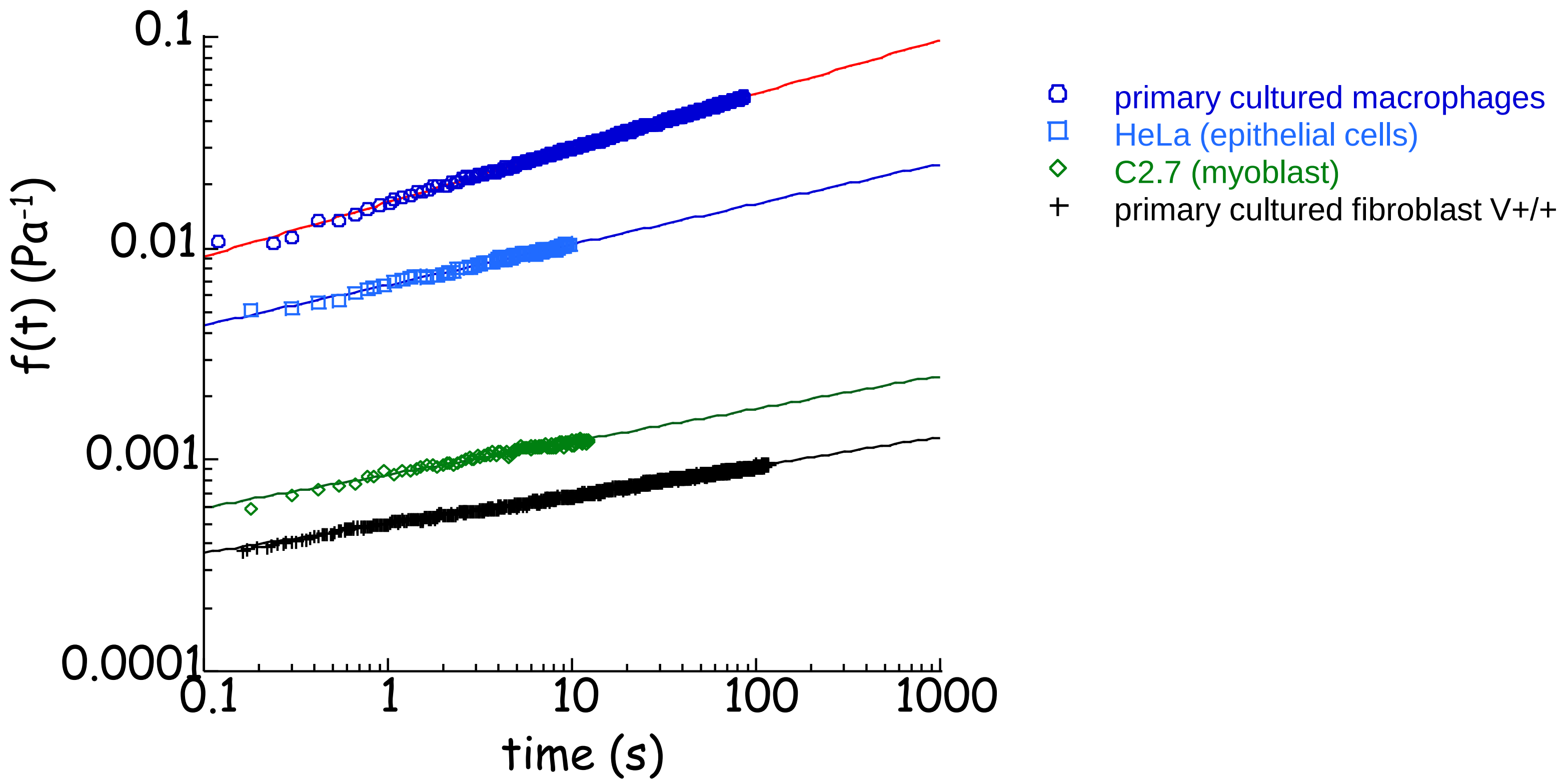
No characteristic time





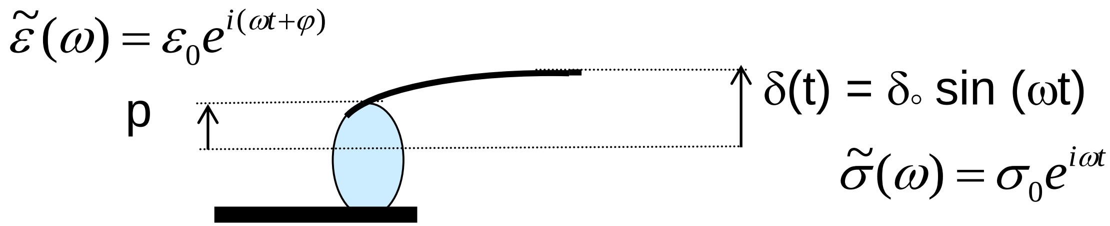


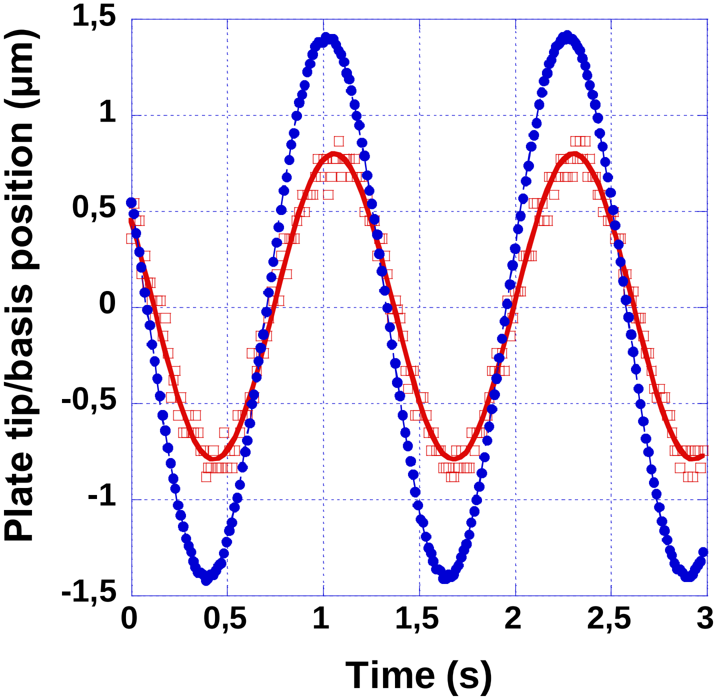
« Universal« behavior

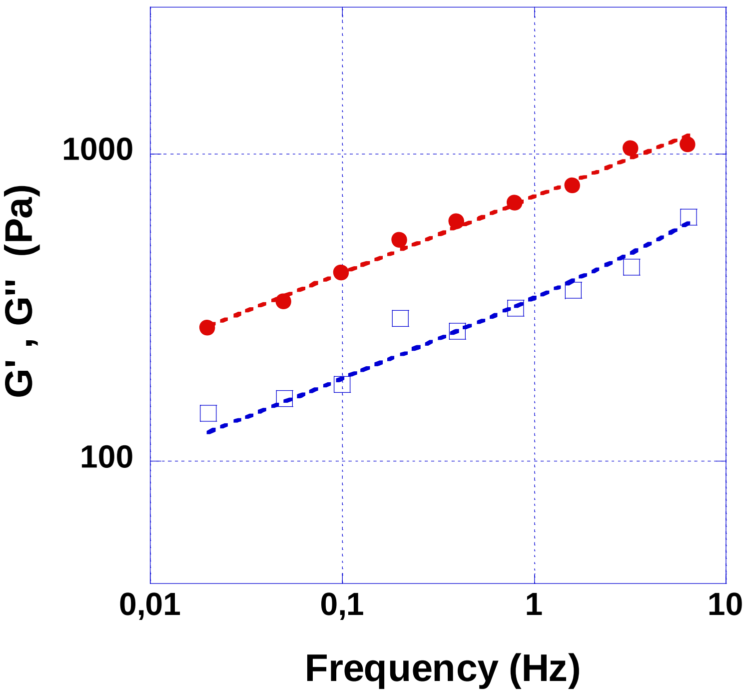


cancer cells F9, J774 alveolar macrophages, A549 alveolar epithelial cells, BEAS-2B of bronchi, human neutrophiles

Viscoelastic modulus at small strains







Power law behavior is consistent

Linearity at large strains

Desprat et al., Rev.Sci. Instrum. ***77****, 055111-1 (2006)*

Derivations

The fundamental relation of **linear viscoelasticity**

$$\varepsilon(t) = J(t)\sigma(0) + \int_0^t J(t-t')\dot{\sigma}(t')dt'$$

Then becomes

$$\varepsilon(t) = J(t)\sigma(0) + \int_0^t J(t-t')\sigma(0)\dot{\varepsilon}(t')dt'$$

Laplace transform then yelds

$$F(p) = \mathcal{L}\{f(t)\} = \int_{0^-}^{+\infty} e^{-pt} f(t)\, dt.$$

$$\tilde{\varepsilon}(s) = \frac{\sigma(0)\tilde{J}(s)}{[1 - s\sigma(0)\tilde{J}(s)]}$$

Assuming that $J(t) = At^{\alpha}$ **as measured in the creep regime**, one finds

$$\varepsilon(t) = \sum_{n=1}^{+\infty} \frac{[\Gamma(1+\alpha)\sigma(0)\mathrm{A}t^{\alpha}]^n}{\Gamma(1+n\alpha)}$$

Thus, at high strains, deformation should well be described by a sum of integer powers of the creep function *J(t)*

Soft Glassy Material or … Fractal Gel

AFM: **L~30 nm** $\alpha \sim 0{,}20$; $G_0 \sim 710$ Pa (*Alcaraz et al., Biophys J., 2003*)

MTC; OT: **L~3 µm** $\alpha \sim 0{,}20$; $G_0 \sim 300$ à 3000 Pa (*Fabry et al., Phys Rev E., 2003*)

(*Balland et al., E. Biophys. J., 2005*)

In agreement with measurements at the cellular scale L~30 µm

$$J(t) = A.t^{\alpha} \xrightarrow{\text{T.F}} G'(f) = \frac{(2\pi)^{\alpha}\cos(\alpha\frac{\pi}{2})}{A\Gamma(1+\alpha)} f^{\alpha}$$

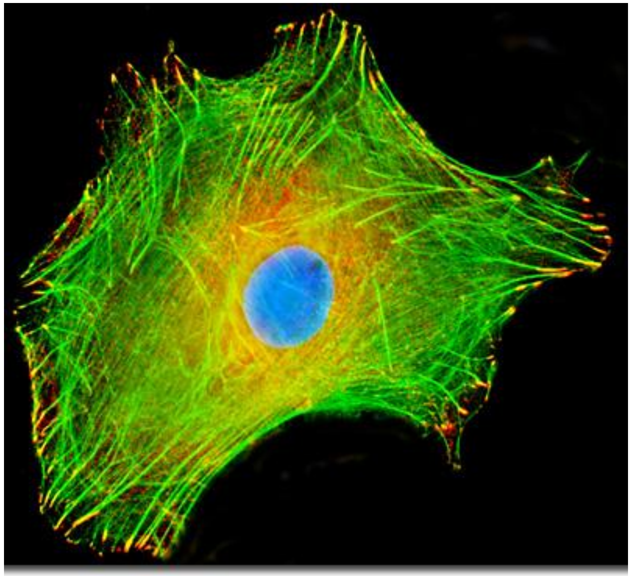
$G_0 = 660$ Pa

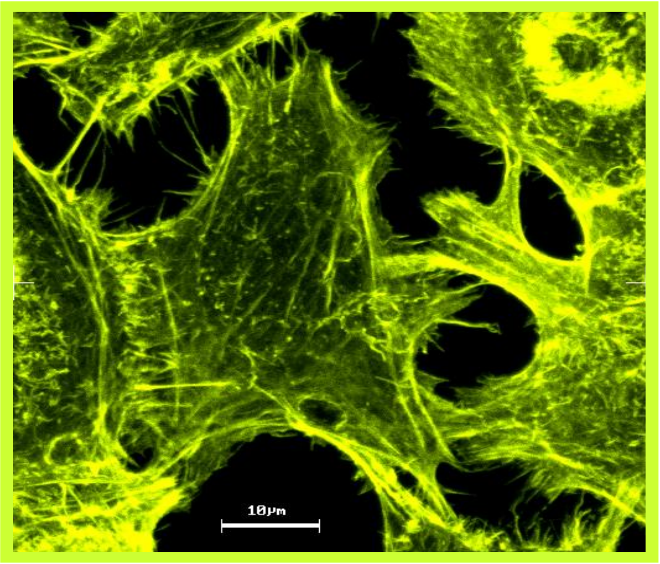
Auto-similarity ?

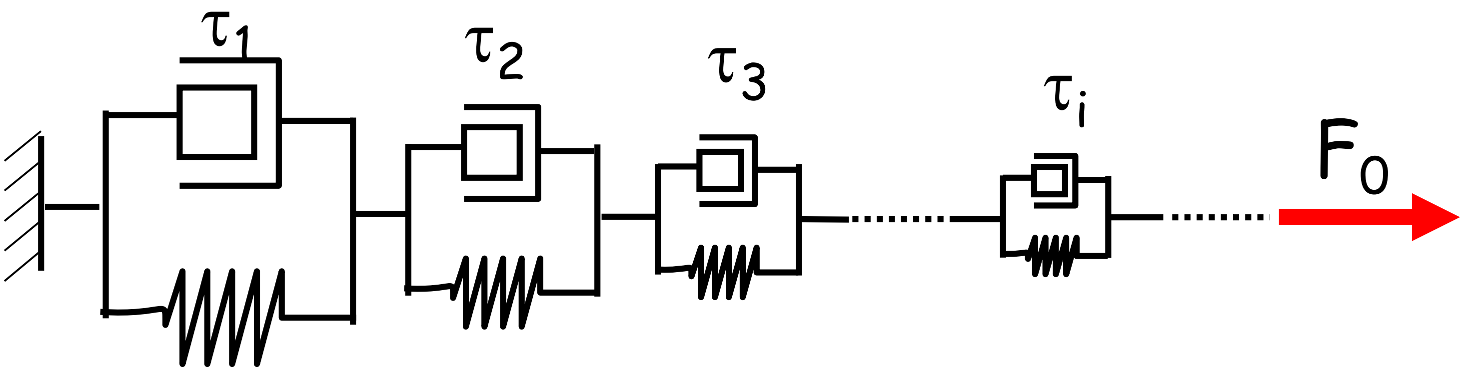
A simple constitutive model

Actine network:
- individual filaments
- bundles
- fibers

unevenly distributed in the cell body







The actin network is modeled by an infinite series of nested elementary viscoelastic units with a wide distribution *p*(τ) relaxation times τ

Distribution of response times

Balland et al., Phys.Rev.E ***74****, 021911 (2006)*

Simple assumptions:

- N(d) number of units of size d
  N(d) ~ d^{-a} if self similar structure

- relaxation time linked to spatial scale: $\tau \sim d^b$

Then **$p(\tau) \sim \tau^{\alpha-2}$** with $\alpha = 1 - a/b$

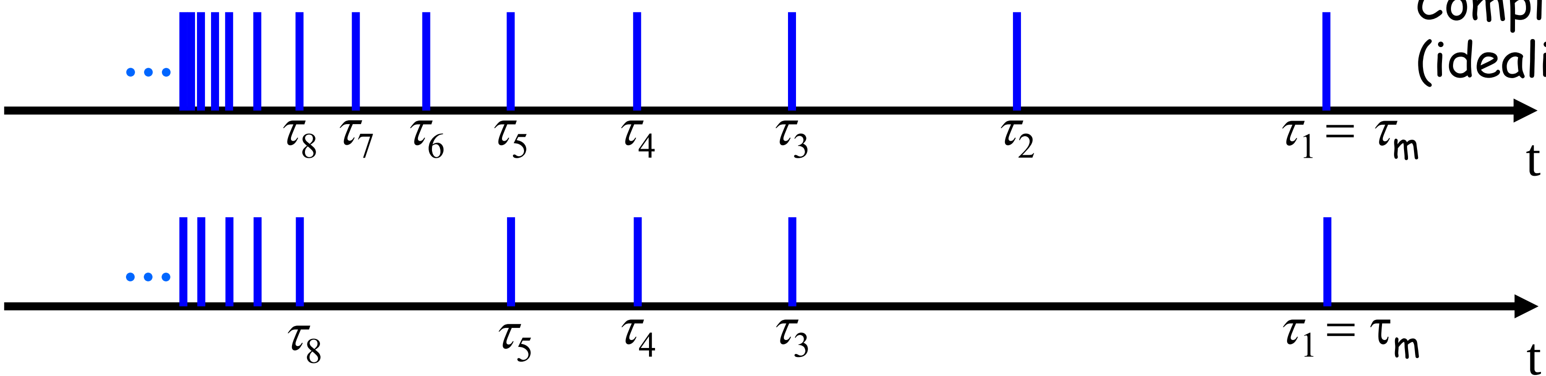
$p(\tau) \sim \tau^{\alpha-2}$ in power law ➡ creep function J(t) as well

$$\frac{dJ}{dt} = \sum_{i=1}^{\infty} \exp\left(-\frac{t}{\tau_i}\right) \approx \int_0^{\infty} \tau^{\alpha-2} \exp\left(-\frac{t}{\tau}\right) d\tau \propto t^{\alpha-1}$$

so **$J(t) \sim t^{\alpha}$**

Agreement with experimental observations

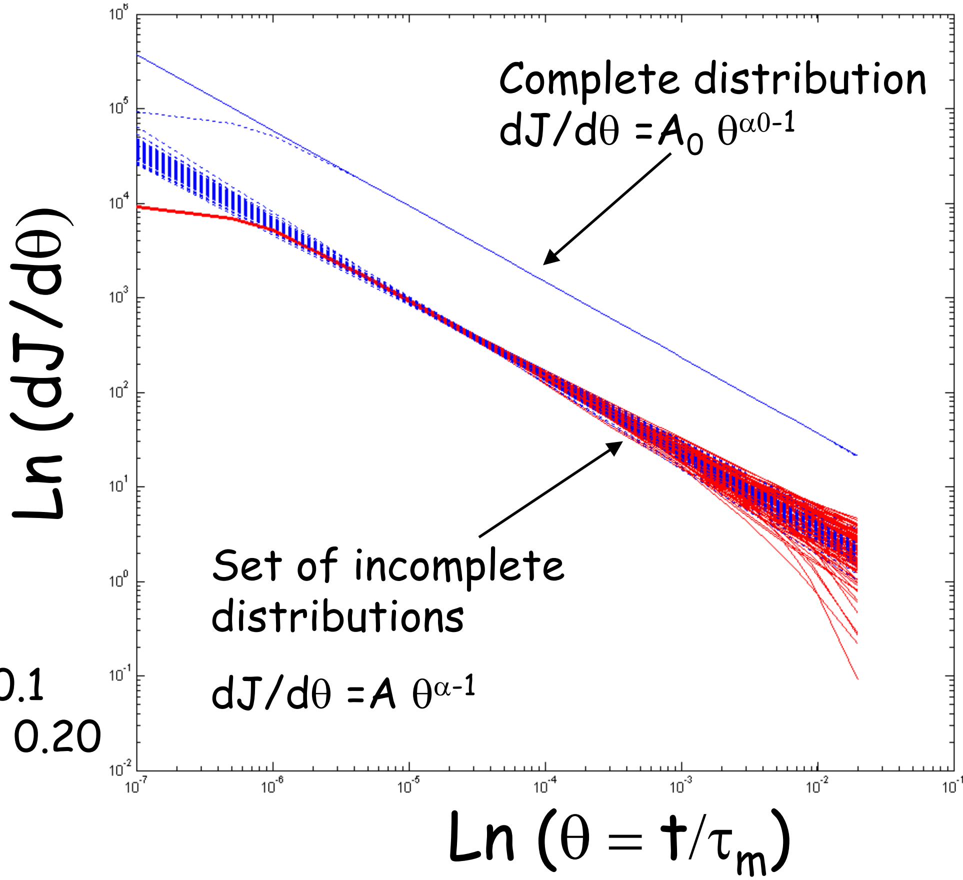
Response of the system



Complete distribution (idealized)

Incomplete distribution: we randomly keep a fraction s of the elements

(Simulates the variability from one cell to another)



$s = 0.1$

$\alpha_0 = 0.20$

$dJ/d\theta = A\ \theta^{\alpha-1}$

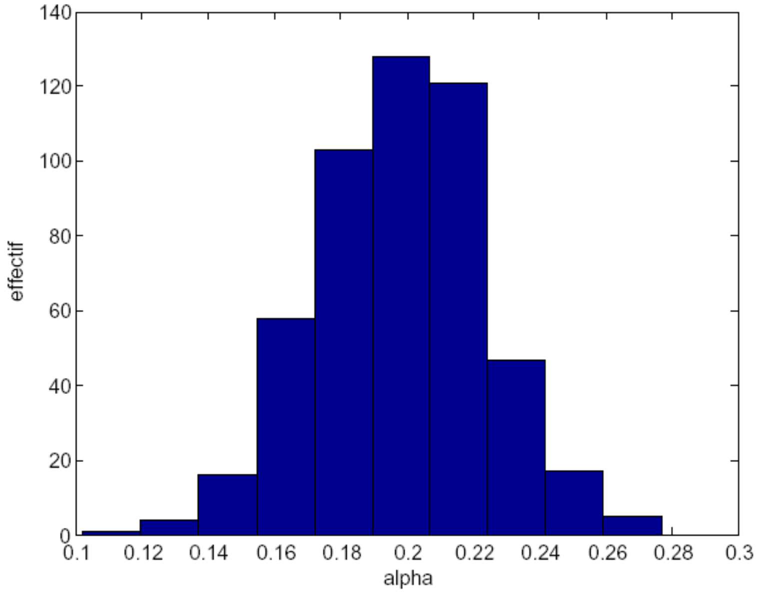
$J(\theta) = (A/\alpha)\ \theta^{\alpha}$

Dispersion of coefficients of the power law

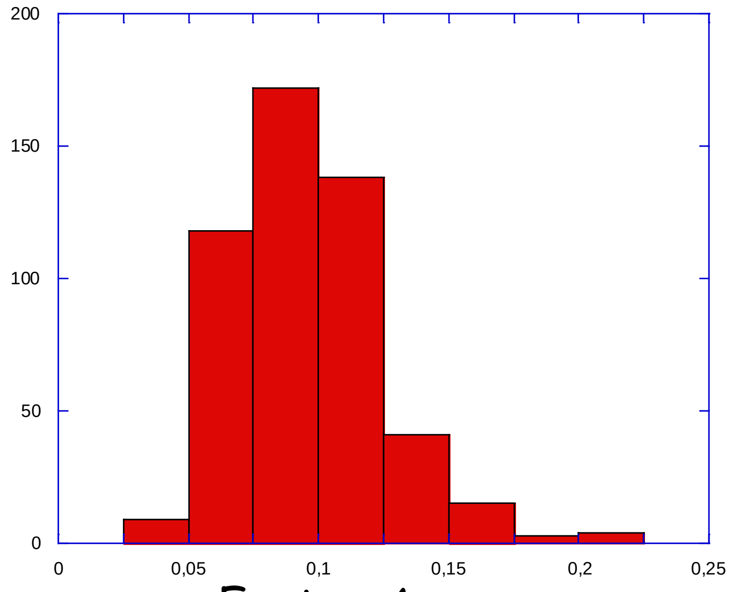
$J(\theta) = A\ \theta^{\alpha}$

- Normal distribution of exponents α
- Log-normale distribution of factors A

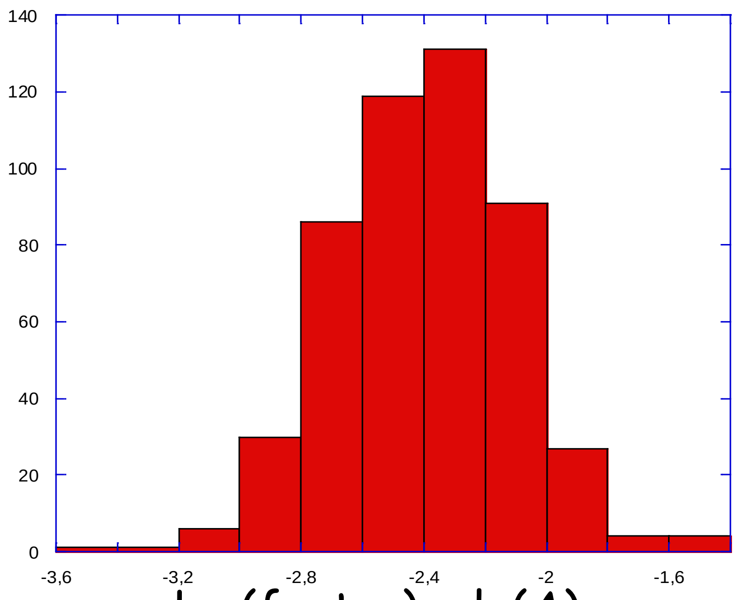
cf experimental results



Exponent α



Factor A



Ln (factor) = ln(A)

Soft Glassy Material or …

Like foams, emulsion, sluries

Desordered medium with a great number of elements and **out of equimibrium**

Interaction between mesoscopic elements leads to

→ large distributions of sizes and relaxation times:
no characteristic time scale

→ specific relaxation processes :
non viscous dissipation

Parameter of control **x (noise temperature)**

→ **power law** rheological behaviour, $\alpha = x - 1$

Possible origins of the power law behavior

foams, emulsions, pastes, slurries

- Out of equilibrium
- Permanent structural rearrangement

↓

<u>Soft Glassy Materials (SGM)</u>

Sollich, ***Phys. Rev. E*** (1998)

⇩

Dynamic origin

Partially polymerized gels

- Fixed structure
- Fractal dimension

↓

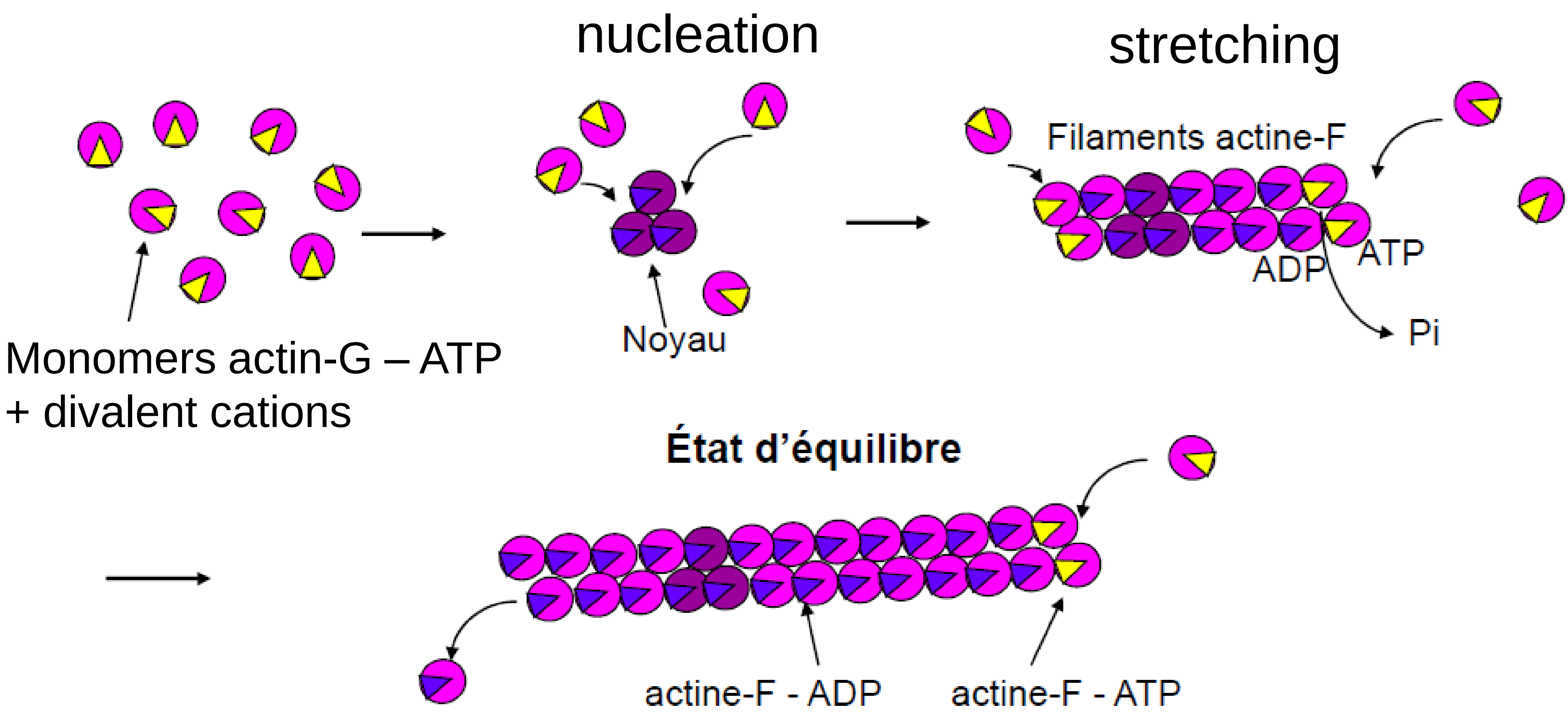
<u>Materials at the « Sol-Gel » transition</u>

Winter *et al.*, ***J. of Rheology*** (1986)

⇩

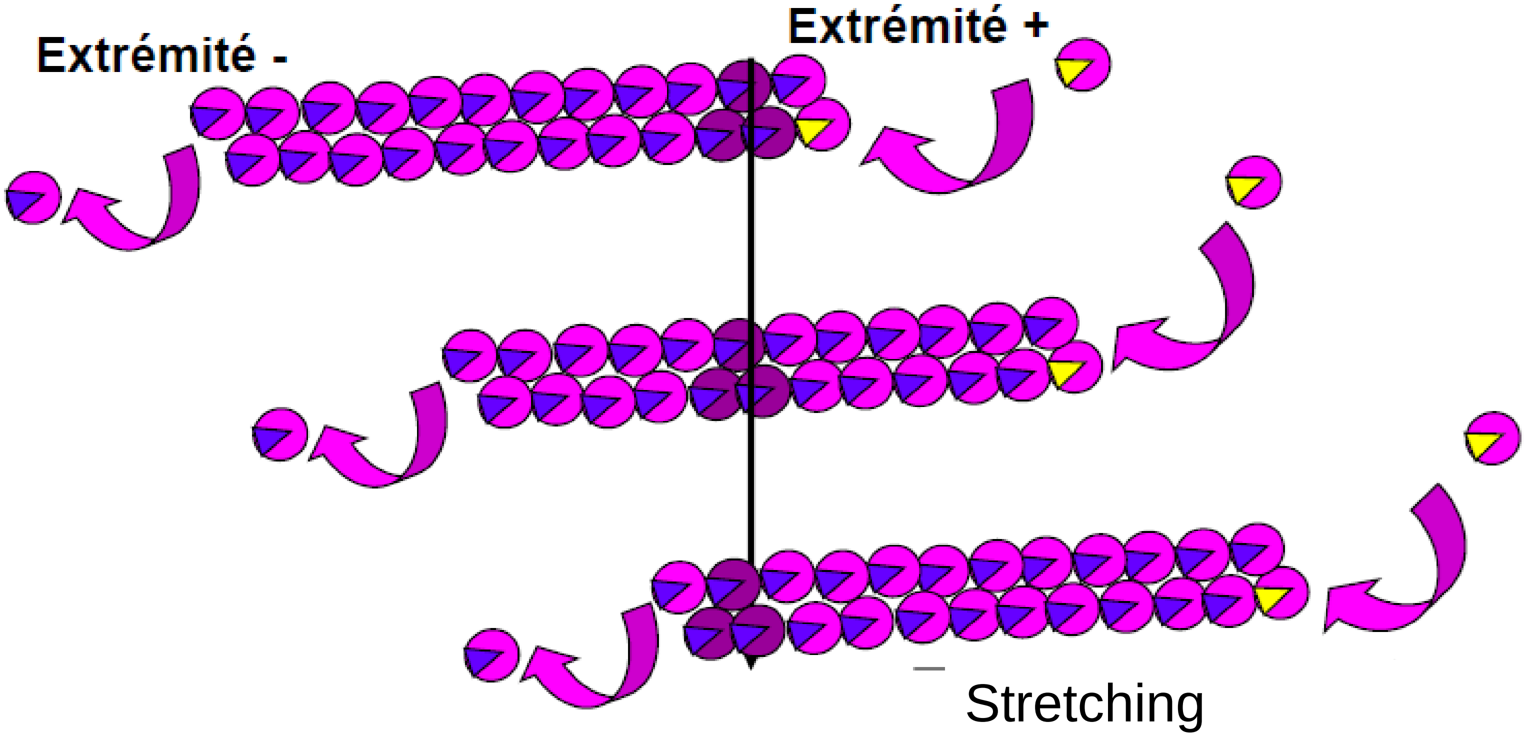
Structural origin

POLYMERIZATION OF ACTIN FILAMENTS

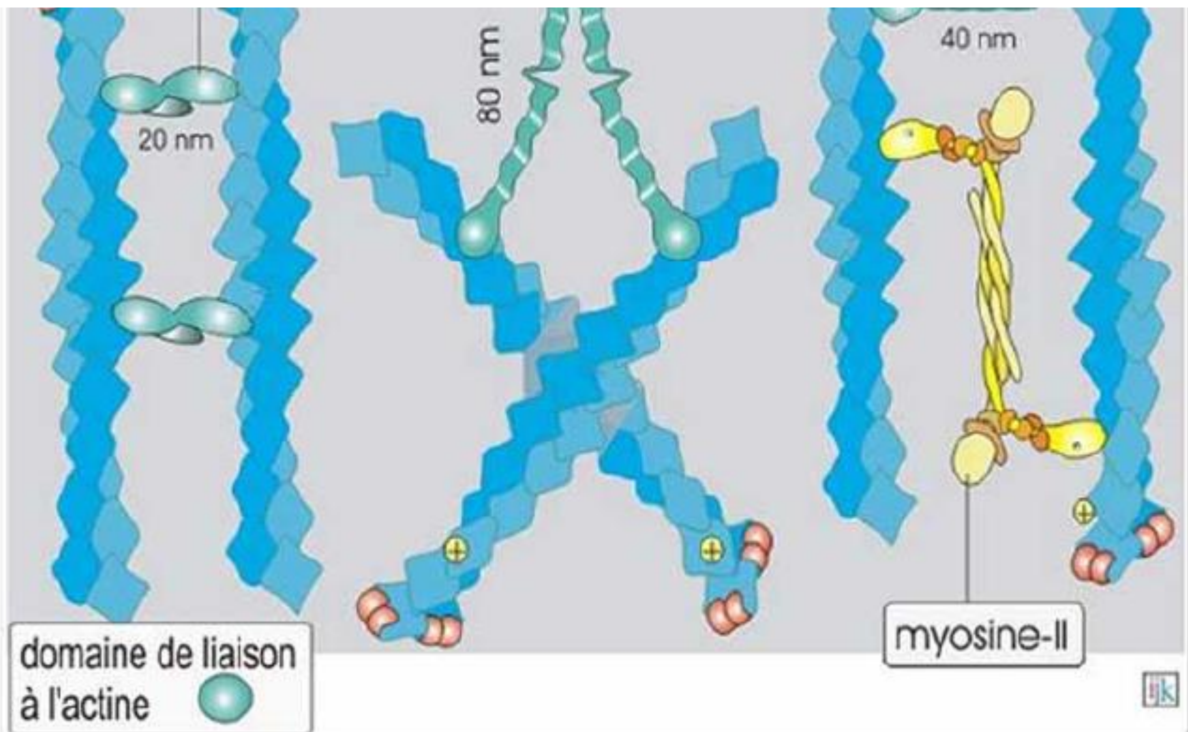


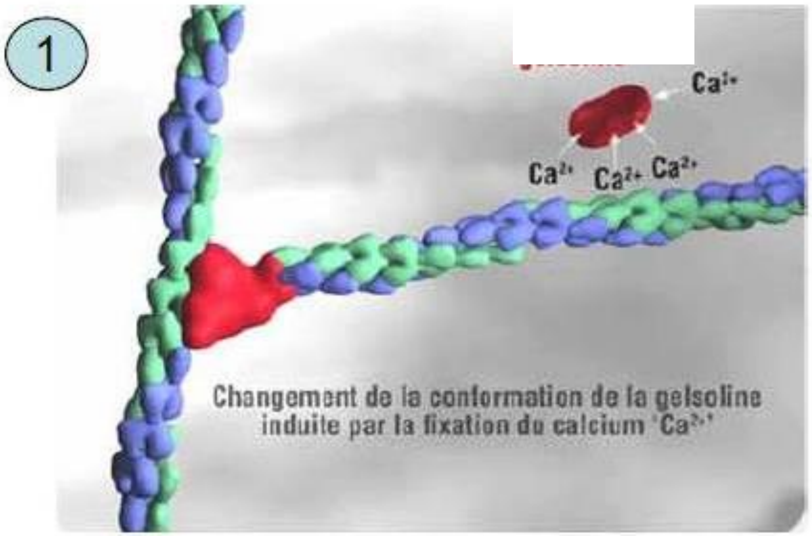
treadmilling

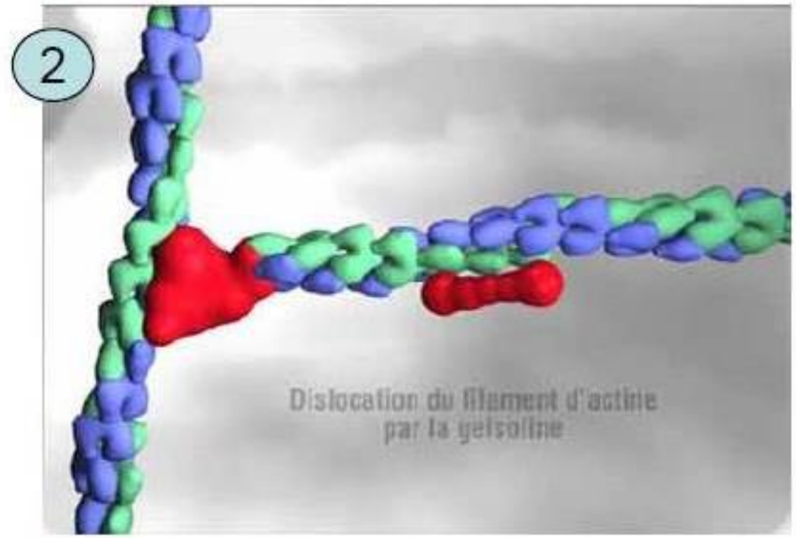
Cc- (0,8µM)> C actine-G > Cc+ (0,1µM)

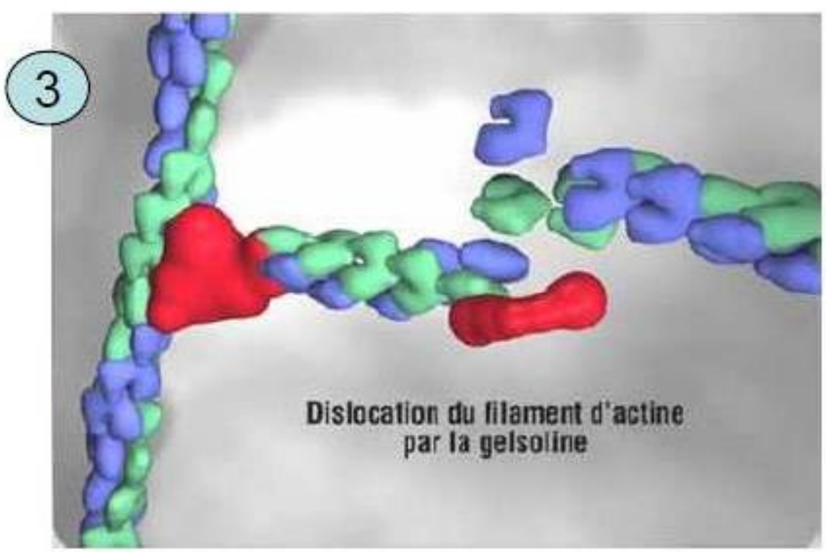


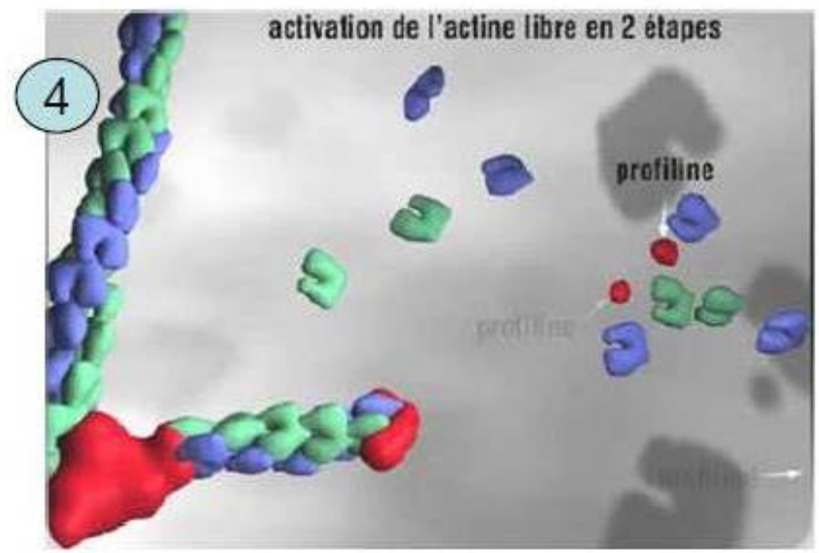
STRUCTURE OF ACTIN FILAMENTS IN THE CELL




1
Ca²⁺
Ca²⁺ Ca²⁺ Ca²⁺
Changement de la conformation de la gelsoline
induite par la fixation du calcium 'Ca²⁺'


2
Dislocation du filament d'actine
par la gelsoline


3
Dislocation du filament d'actine
par la gelsoline


4
activation de l'actine libre en 2 étapes
profiline
profiline