Biomechanics of bladder cancer cells

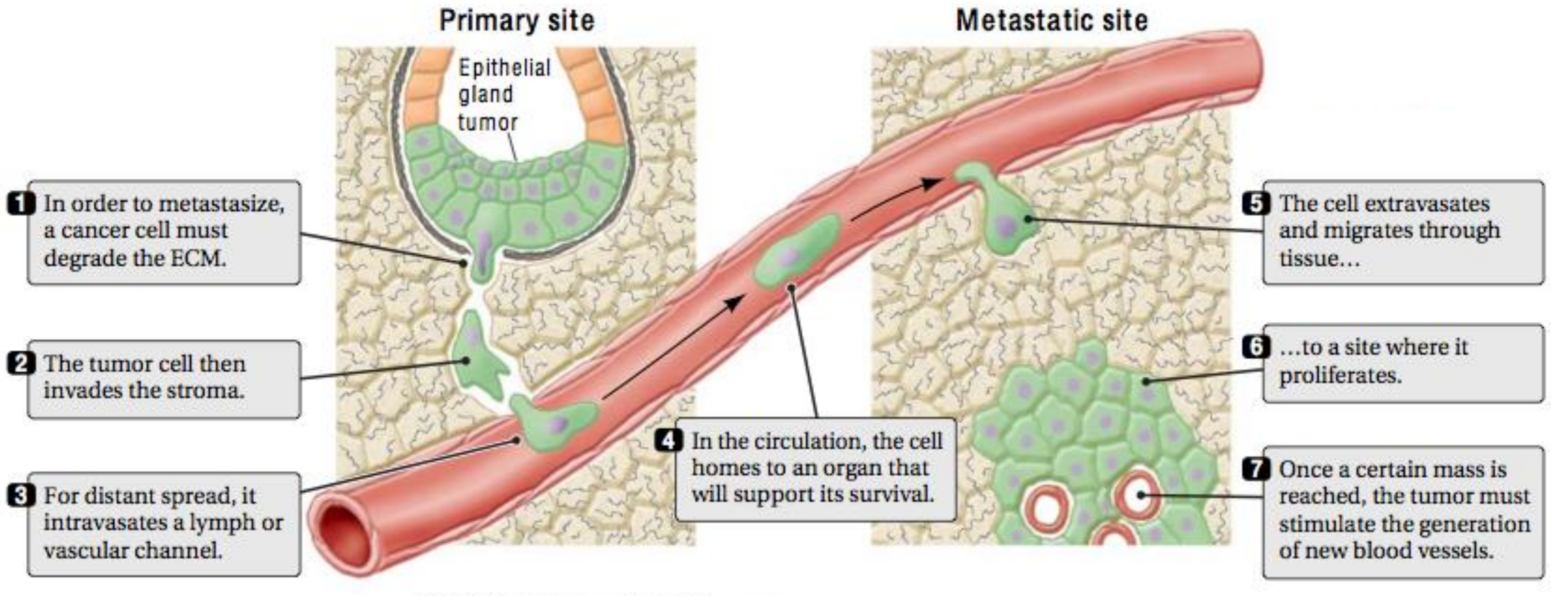
– what we have learned over two decades of studies

Małgorzata Lekka

The Henryk Niewodniczański Institute of Nuclear Physics

Polish Academy of Sciences

Aims of the study



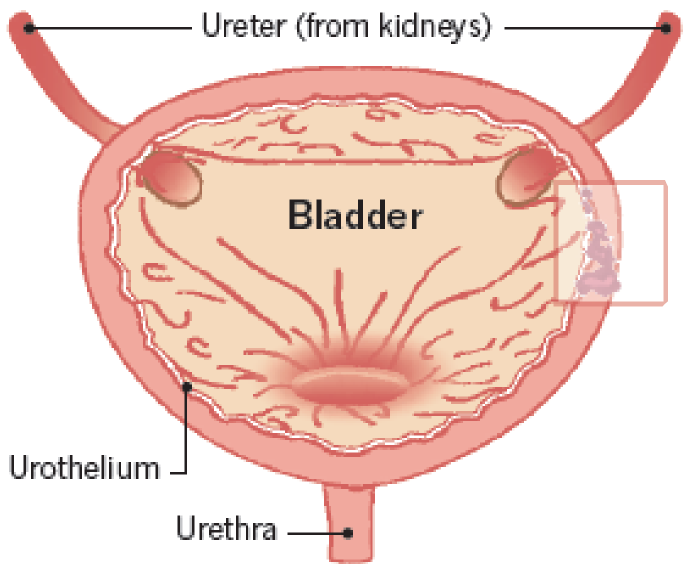
metastasis proceeds through mechanically distinct environments

Geho et al. Physiology 20 (2005) 194

Guimaraes et al. Nat. Rev. Mat. 5 (2020) 351

Mechanical properties of the local cellular microenvironment define the invasive and migratory phenotype of cancer cells through irreversible changes in cell cytoskeleton and cellular surface, leading to softening of cancer cells.

Human bladder cancer



Berdik, Nature 551 (2017) S34

Bladder cancer (BC) is the 12th most frequent cancers in the world and the urological tumor that presents the highest mortality

BC is the most expensive cancer to treat

BC is the most lethal urological malignancy

The most common metastases are:
lymph nodes, lungs, liver, bone, and peritoneum

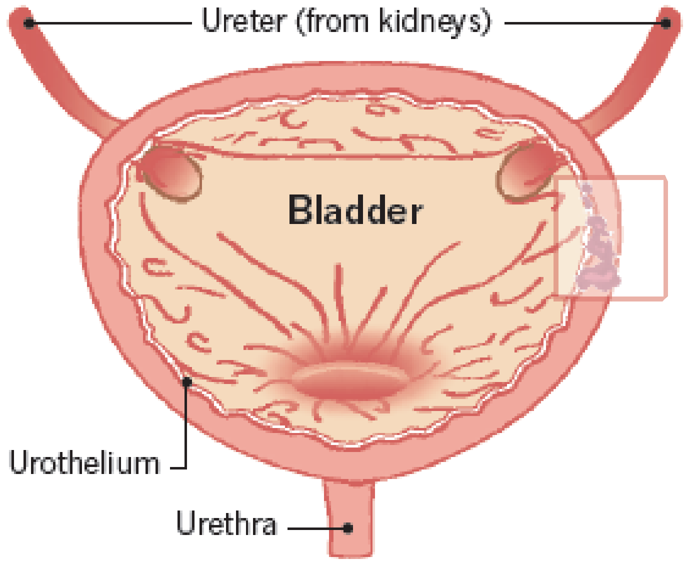
functional stiffness (bladder stretching) ranges from a few kPa to ~100 ÷ 200 kPa

Example pig bladder:

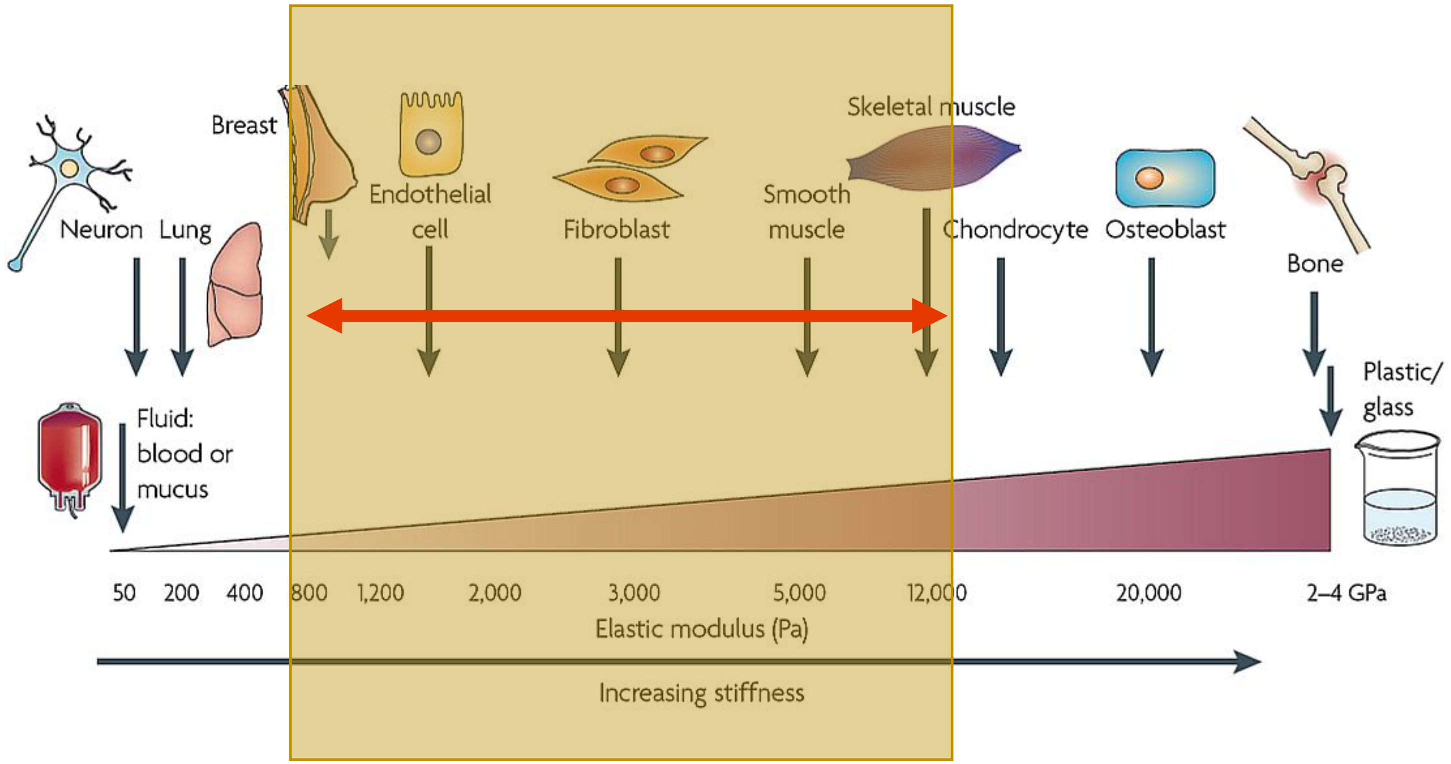
the elastic modulus changes from 9.6 kPa (187 mL, 8.6 mmHg) to 106.9 kPa (327 mL, 27.6 mmHg)

Nenadic et al. Phys. Med. Biol. 58 (2013) 2675

Human bladder cancer

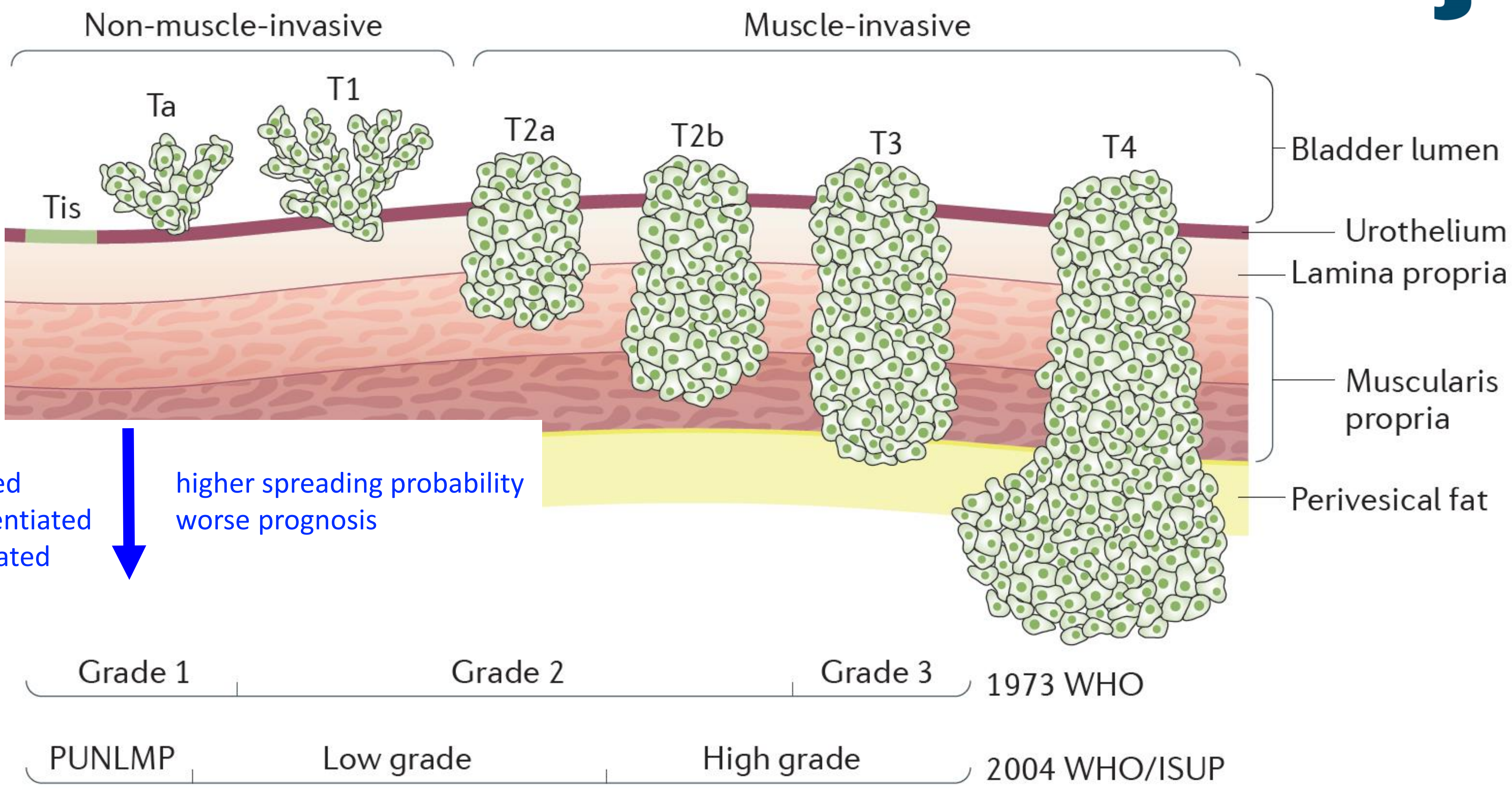


Berdik, Nature 551 (2017) S34



Cox et al. Dis. Mod. Mech. 4 (2011) 165

Human bladder cancer



Grades:
G1: well differentiated
G2: moderate differentiated
G3: poorly differentiated

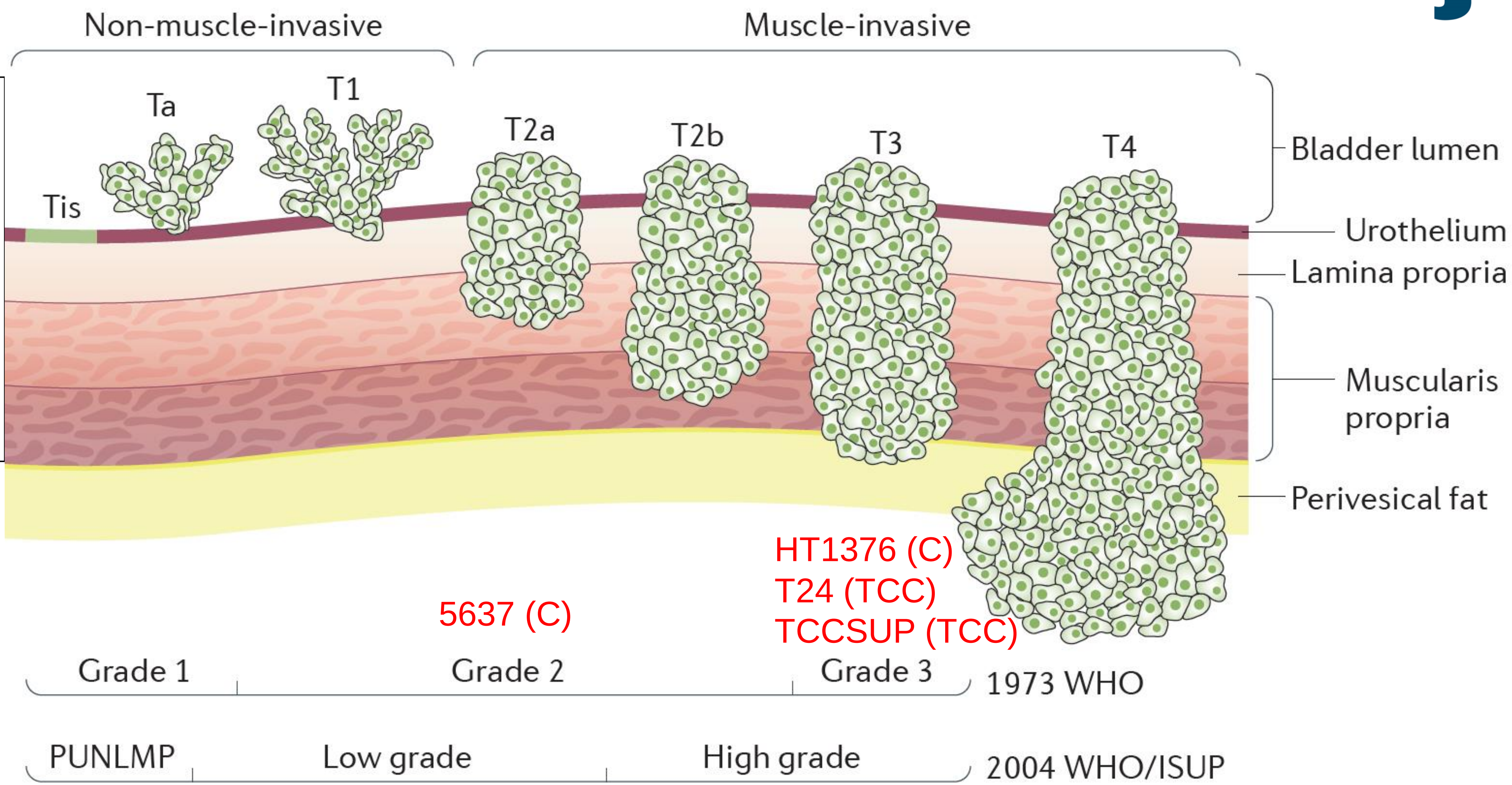
higher spreading probability
worse prognosis

Sanli et al., Nat. Rev. 3 (2017) 17022

Human bladder cancer

HCV29 cells – non-malignant cell cancer of the ureter, derived from non-malignant part of the irradiated, cancer-free bladder transitional epithelium of the patient suffering from bladder carcinoma

O'Toole et al., J. Exp. Med. 139 (1974) 457

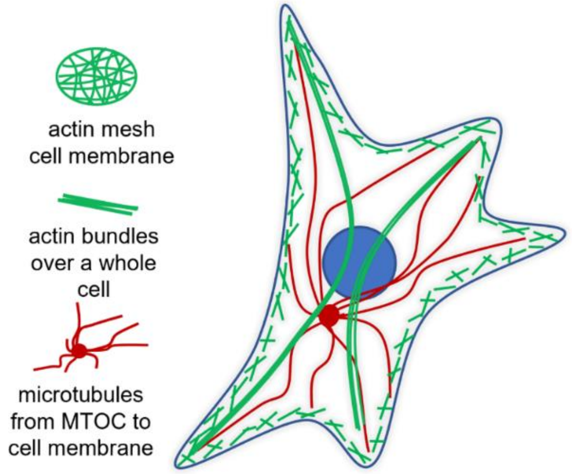


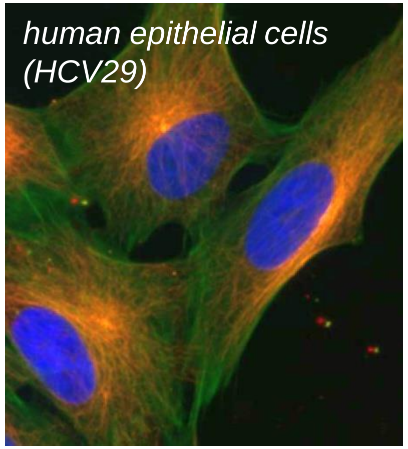
C – carcinoma; TCC – transitional cell carcinoma

Sanli et al., Nat. Rev. 3 (2017) 17022

Cells as viscoelastic materials

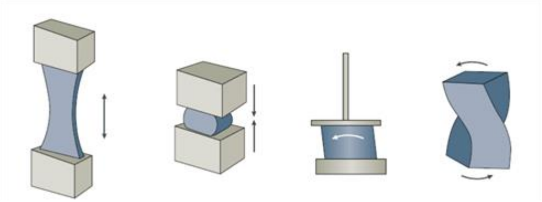
Structure of the cell

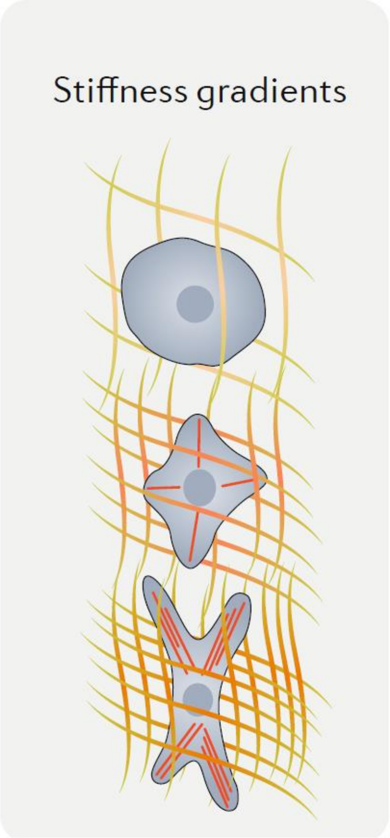




green – actin filaments
blue – cell nucleus
orange – microtubules

How cells are deformed?





Cells are:

- non-isotropic
- not purely elastic but viscoelastic
- adhesive to surrounding microenvironment

Guimaraes et al. Nature Rev. Mat. 5 (2020) 351

Cells as viscoelastic materials

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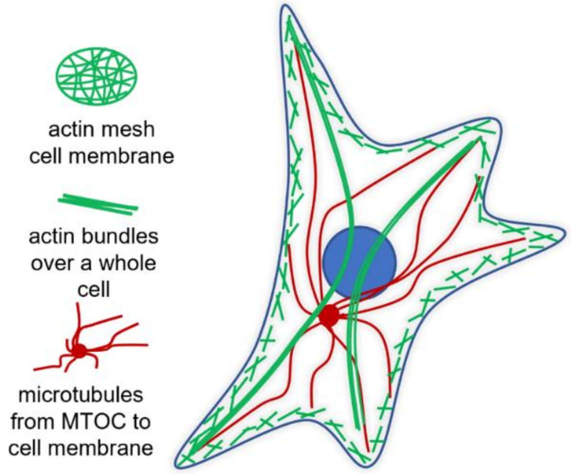

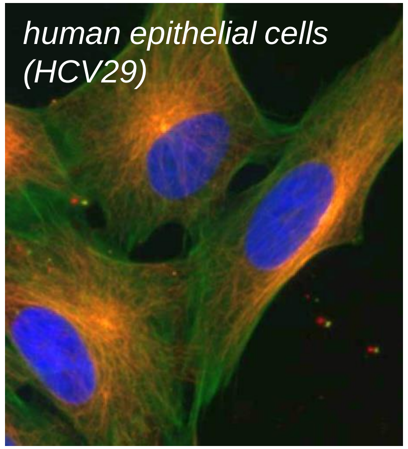

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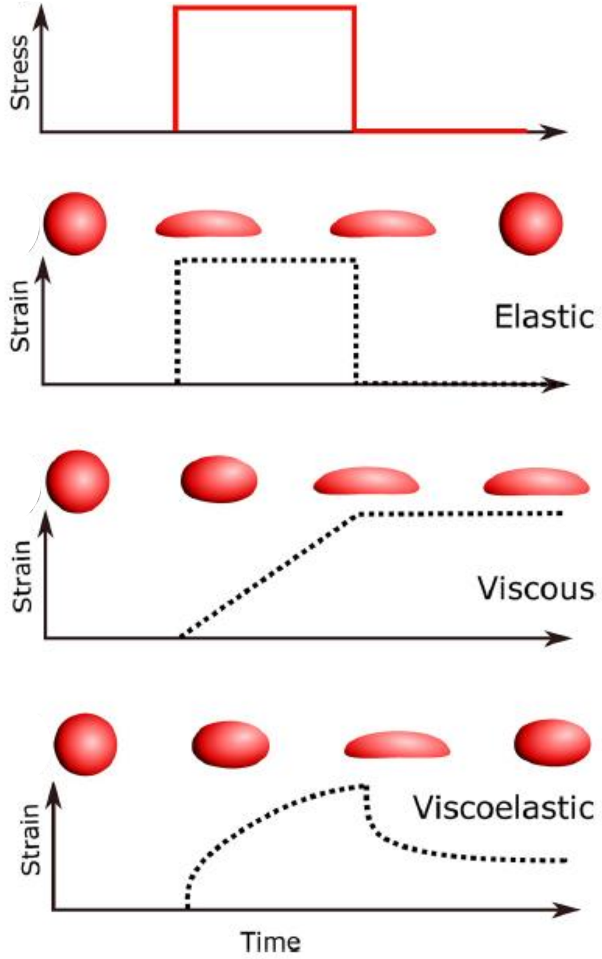

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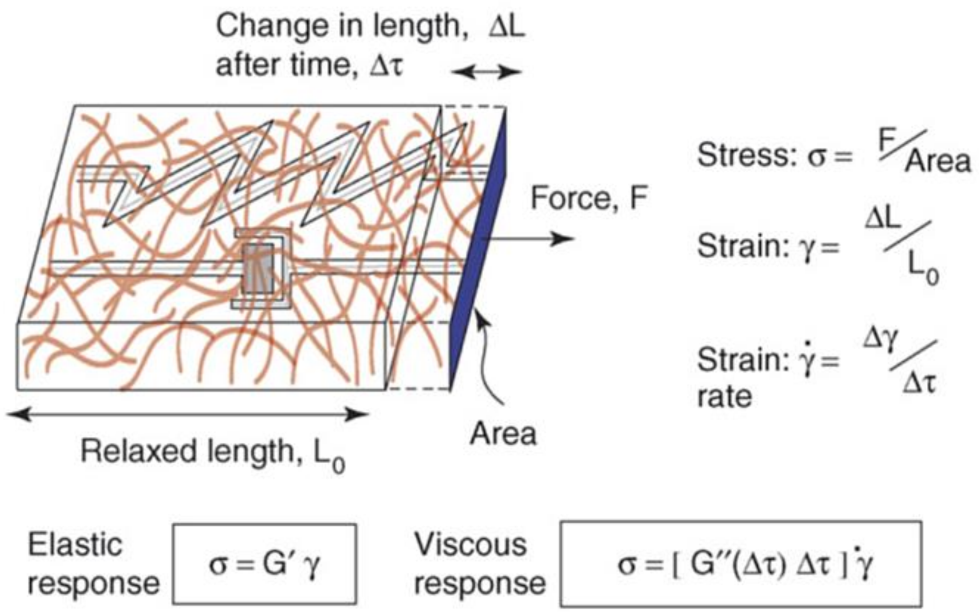
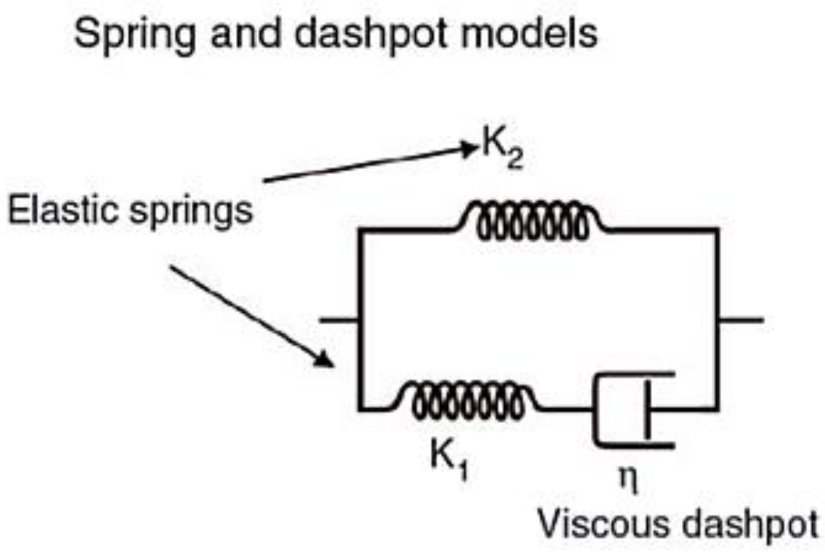
Harris, Sem. Cell. Develop. Biol. 93 (2018) 16

Cells as viscoelastic materials



Kasza et al. Cur. Opin. Cell Biol. 19 (2007) 101

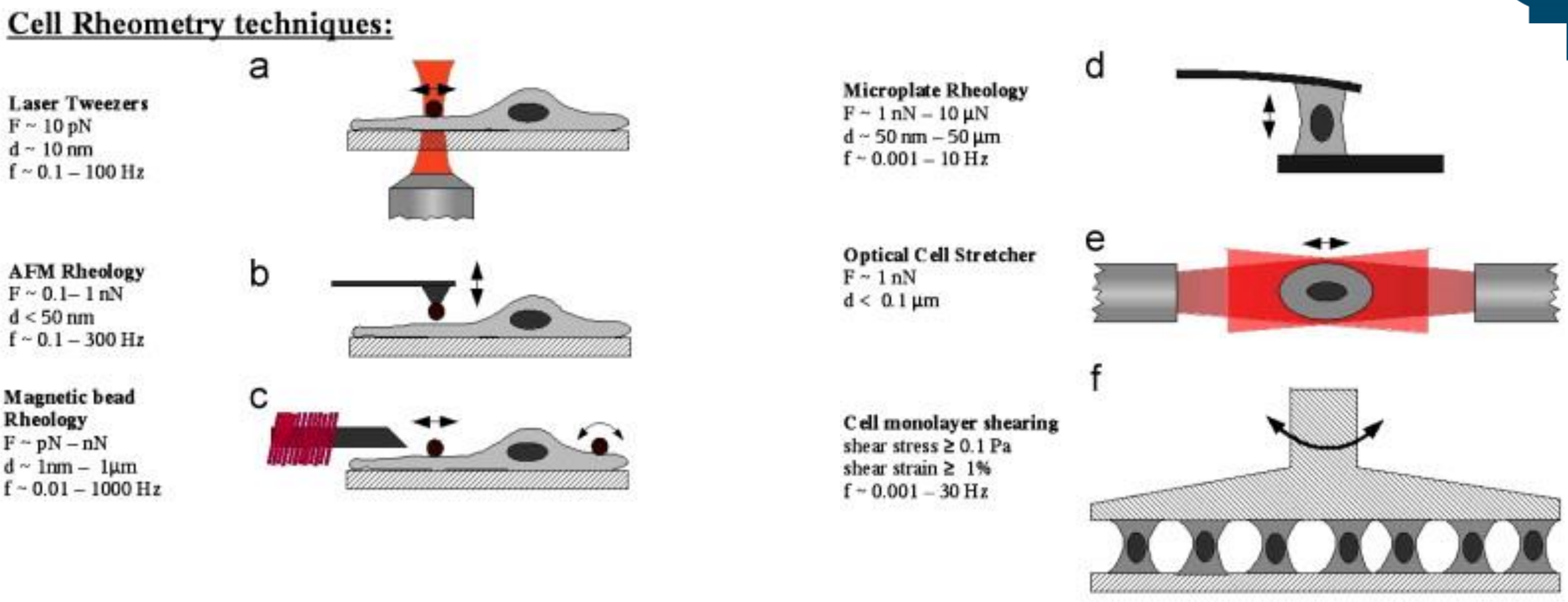


Moeendarbary, Harris,
Wiley Interdiscip Rev Syst Biol Med.
6 (2014) 371

- *A simple Hookean spring elongates instantaneously when stress is applied. Stress is linear in strain.*

- *Newtonian fluids are characterized by a viscosity (i.e. the ratio between shear stress and shear rate) that is independent of shear rate. These fluids display a linear relationship between shear stress and shear rate.*

Nanoscale measurements of cell biomechanics

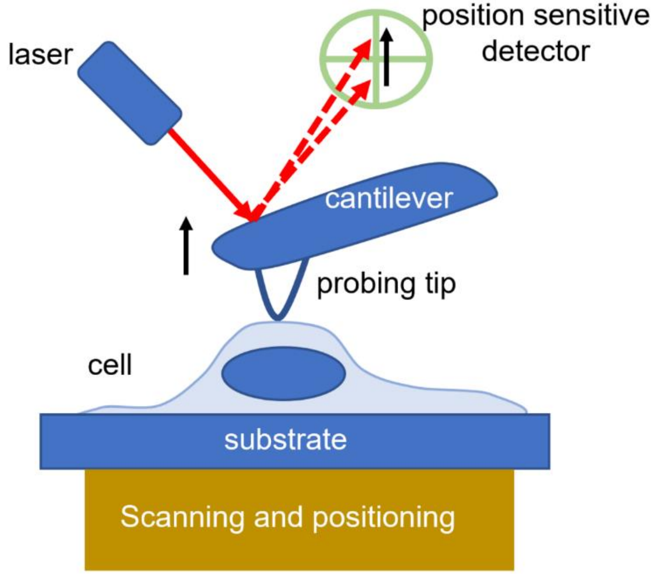


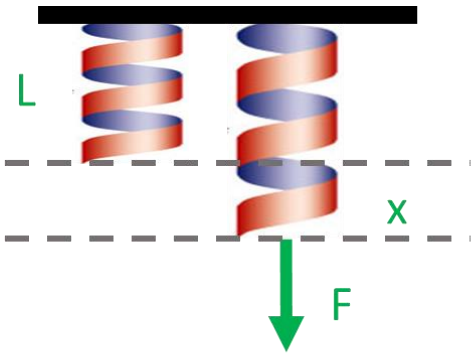
F – applied force
d – deformation
f – frequency range

Pullarkat et al., Physics Reports 449 (2007) 29

Nanoscale measurements of cell biomechanics

Atomic force microscopy



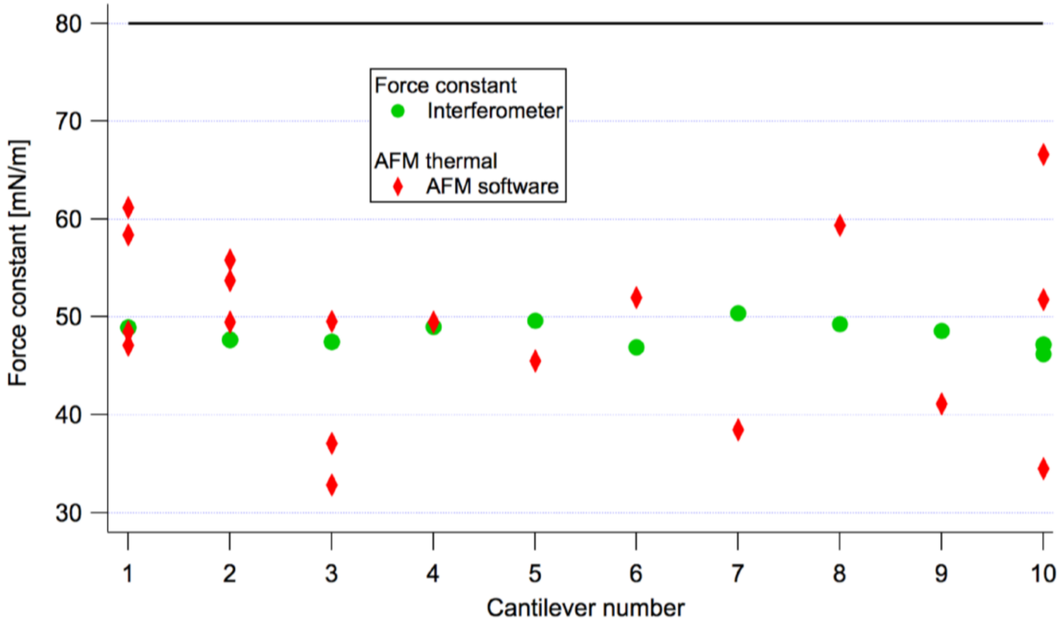


Hooke's law:

$F = k \cdot x$

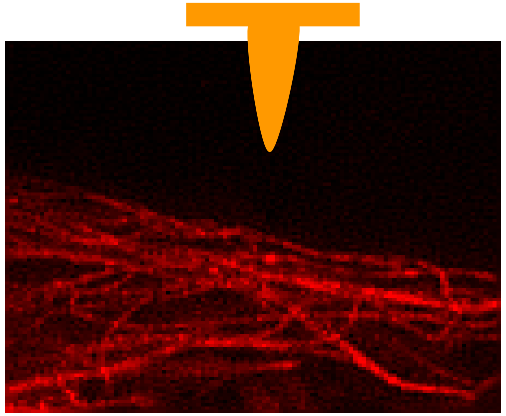
k – cantilever spring constant [N/m]

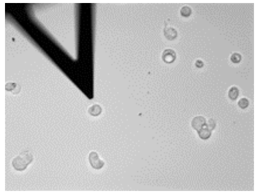
EU COST action TD1002 „European network on applications of Atomic Force Microscopy to NanoMedicine and Life Sciences", 2010 – 2014



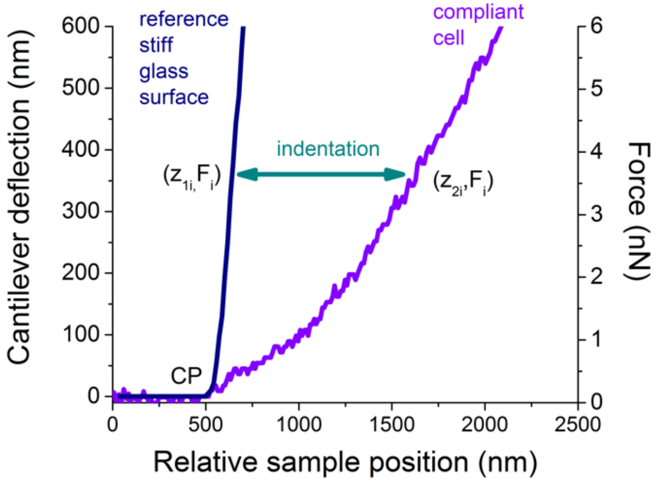
Schillers et al. Sci. Rep. 7 (2017) 5117

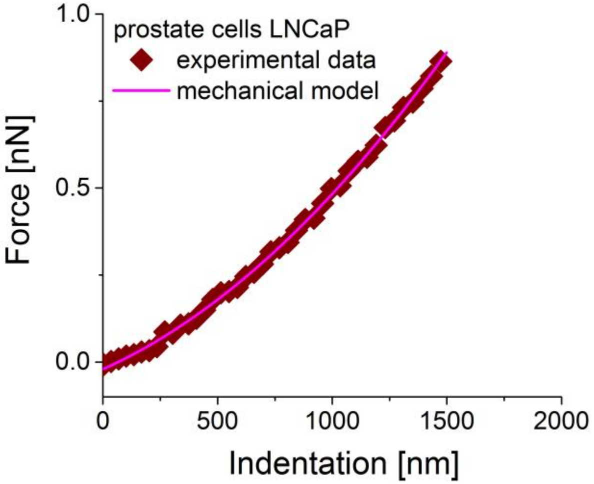
Atomic force microscopy – compression





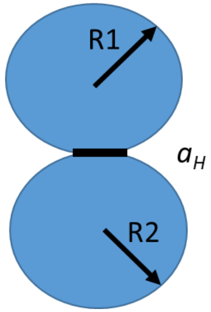
http://www.biocurious.com/images





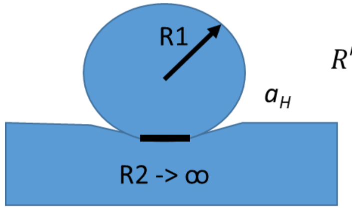
[Hertz contact mechanics (1882)]

- Elastic materials
- No adhesion
- Elastic properties are isotropic



$$\frac{1}{R'} = \frac{1}{R1} + \frac{1}{R2}$$

$$\frac{1}{E'} = \frac{1}{E1} + \frac{1}{E2}$$

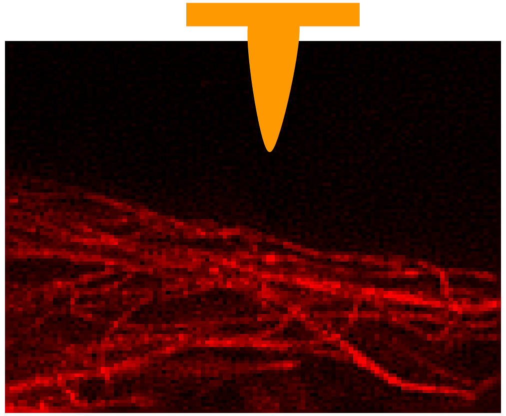


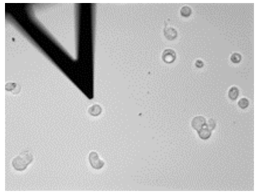
$R' = R1$

$$a_H = \frac{F \cdot R'}{E'}$$

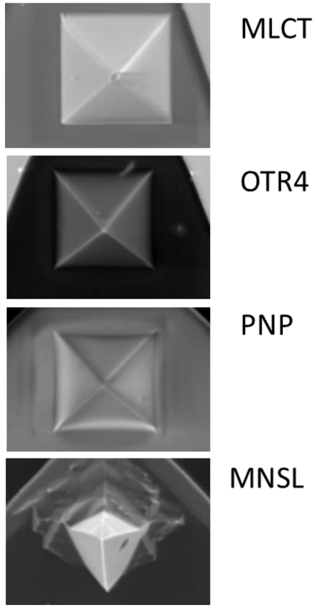
the radius of circle of contact is proportional to the indenter load F, the spherical indenter radius R', and inverse proportional to the elastic properties of the contacting materials, E'.

Atomic force microscopy – compression

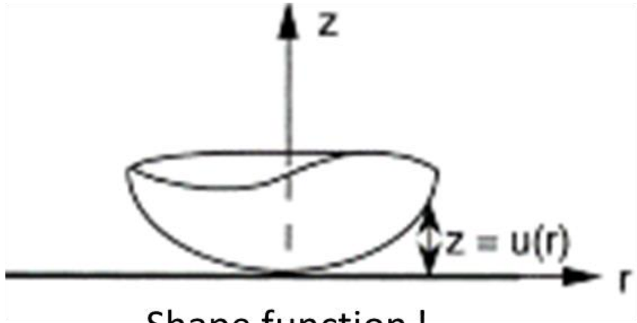




http://www.biocurious.com/images

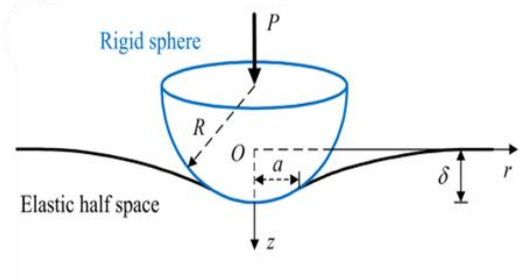


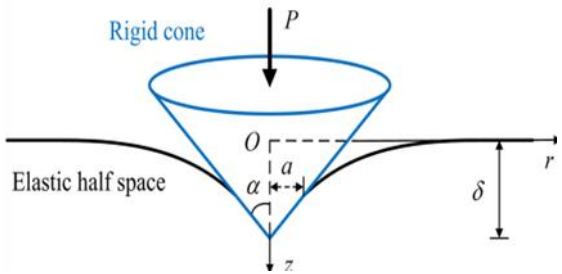
Solving Boussinesq's problem
of symmetric punch
indenting an infinitive half space
(isotropic, purely elastic)



Shape function !

Approximation of the AFM probes





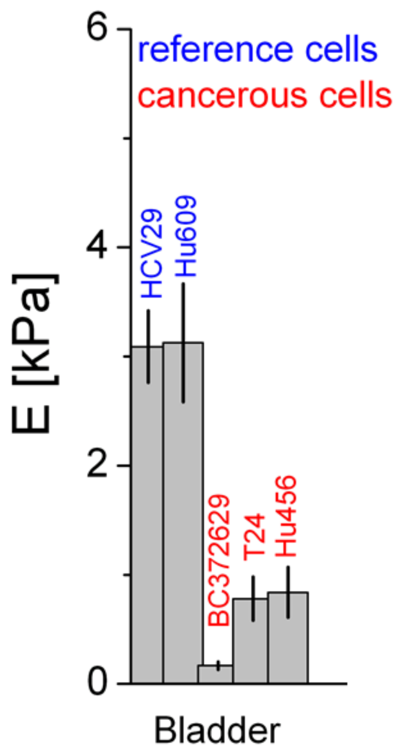
$$F(\delta) = \frac{4 \cdot \sqrt{R}}{3} \cdot E' \cdot \delta^{3/2}$$

$$F(\delta) = \frac{2 \cdot \tan\alpha}{\pi} \cdot E' \cdot \delta^{2}$$

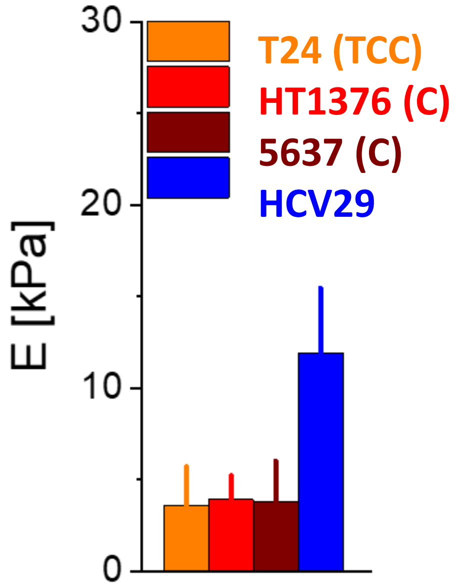
Sneddon, Int. J. Eng. Sci. 31 (965) 47

Long et al. J. Appl. Mech. 84 (2017) 051007

Compressive properties of bladder cancer cells



Lekka at al.,
Eur. Biophys. J.
28 (1999) 312

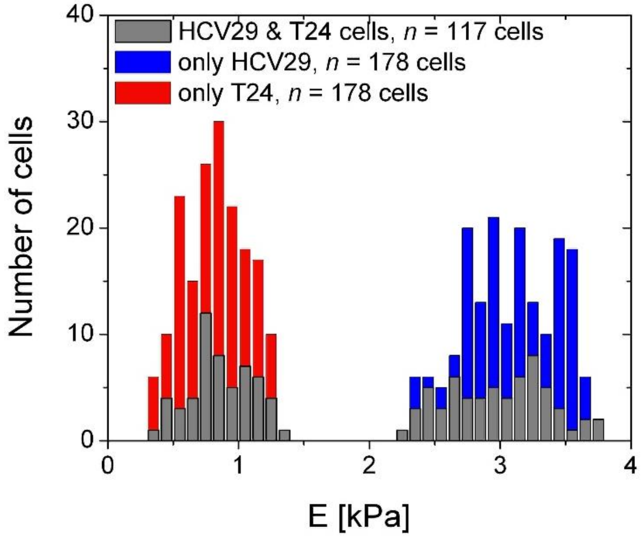


Ramos at al.,
Beilstein. J. Nanotechol.
5 (2014) 447

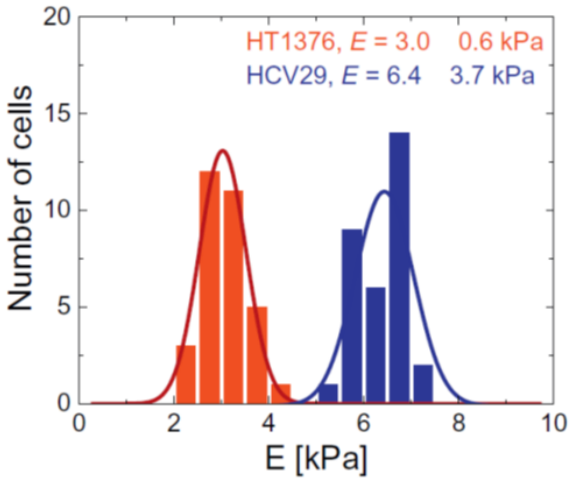
BC3726 - v-raf transfected HCV29 line
Hu456 - TCC

Human bladder cancer cells

- mechanical properties measured in various conditions always show differences between non-malignant and cancerous cells (1999, 2001, 2012, 2014, 2019)
- softening of cancer cells already happens at an early stage of cancer progression



Lekka at al.,
Micron
43 (2012) 1259

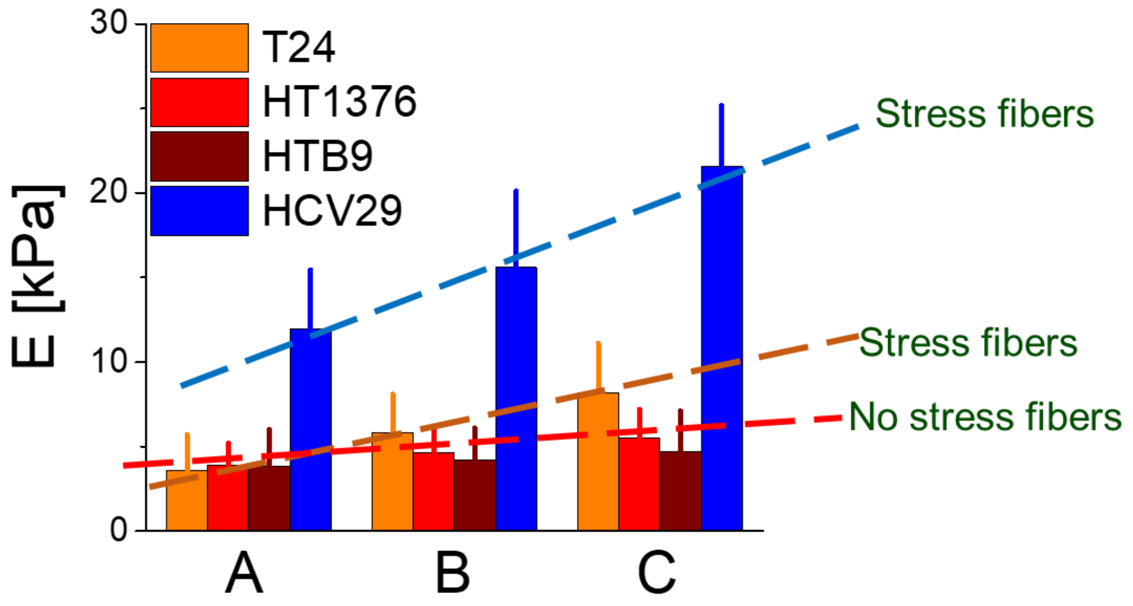


Lekka at al.,
BBA – Gen. Subj.
1863 (2019) 1006

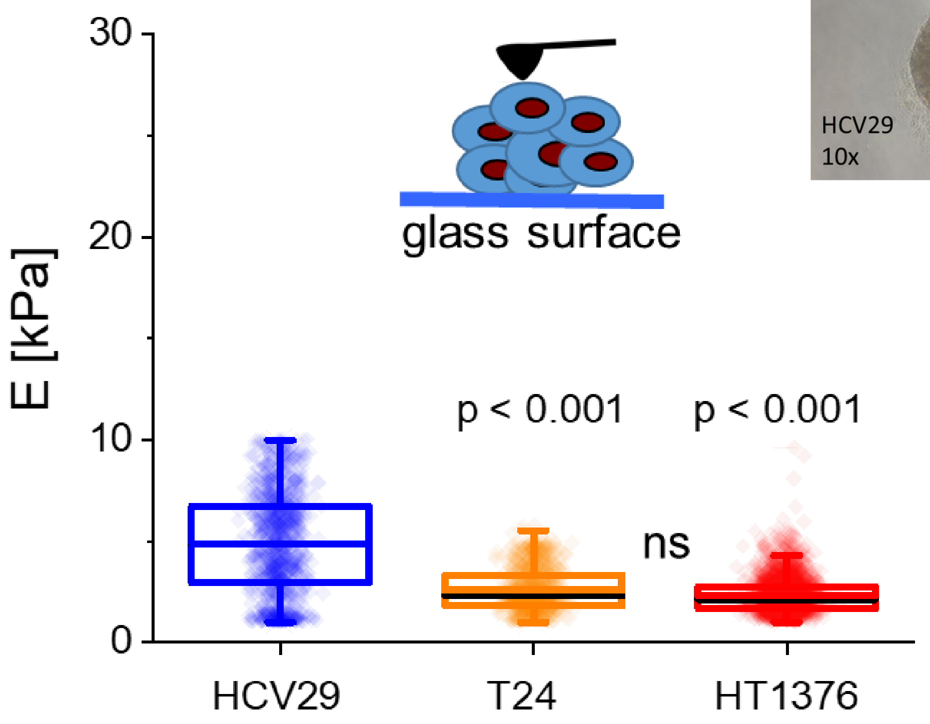
Mechanical properties and organization of cells in bladder tissue

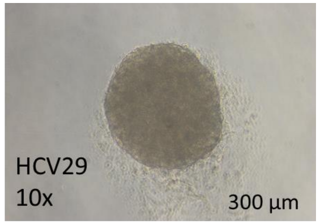
EU H2020- MSCA- ITN 2019-2022
Kajangi Gnanachandran

stress fibers contribute
to the mechanical properties of single cells



A: single cells
B: clusters (5-10)
C: monolayer

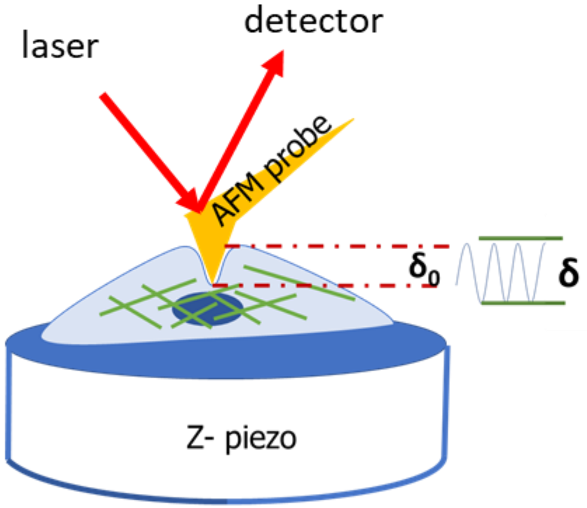




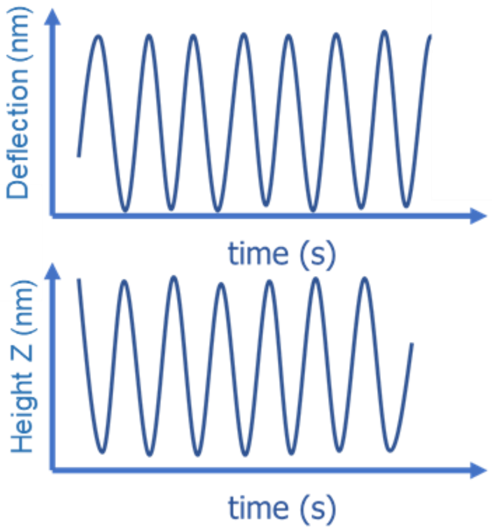
Gnanachandran et al. submitted

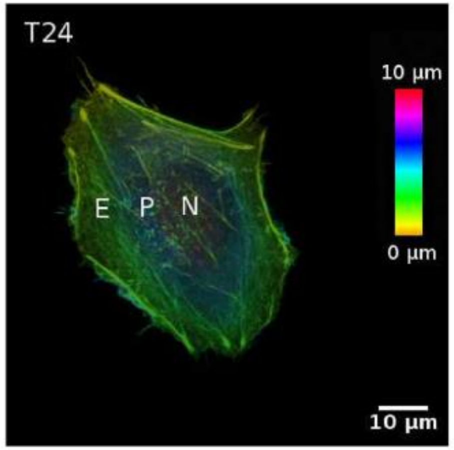
Atomic force microscopy – microrheology

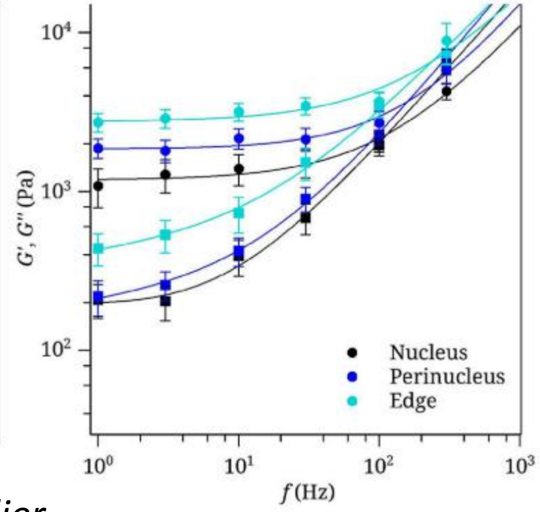
University Grenoble Alpes
Claude Verdier
Joanna Zemła



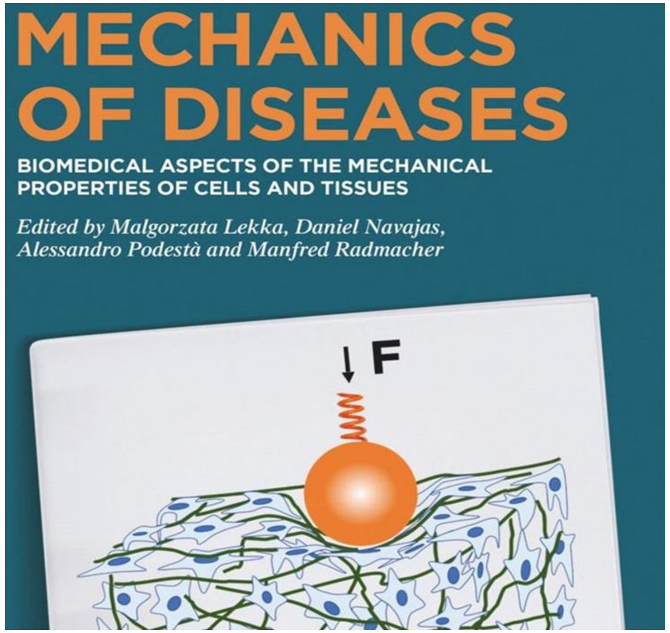
δ_0- initial indentation,
$\delta_0 < 1$ µm







J.Zemla, Y.Abidine, C.Verdier, book chapter in „Mechanics of diseases", 2022



$$E^* = \frac{2\,(1-\nu^2)}{3\,\delta_o\,\tan\theta}\,\frac{F\,(\omega)}{\delta\,(\omega)}$$

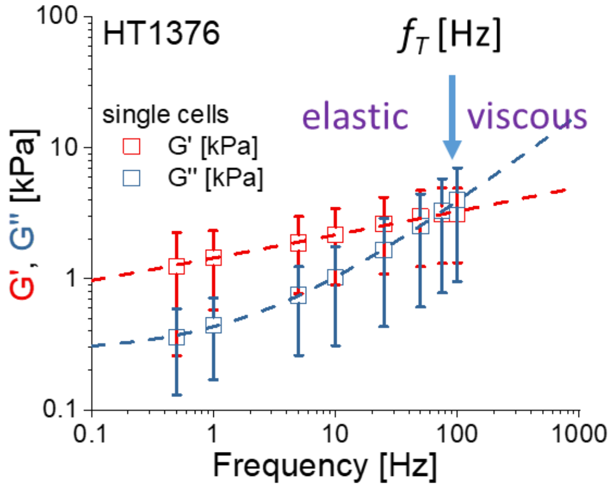
$$G^* = \frac{E^*}{2(1+\mu)}$$

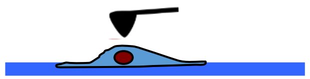
Alcaraz et al. Biophys. J. 84 (2003) 2071
Abidine et al. Eur. Phys. J. Plus, 130 (2015) 202

Rheological properties of bladder cancer cells

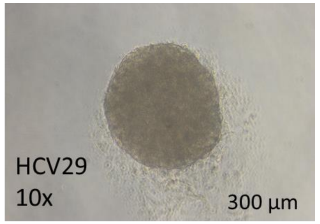
EU H2020- MSCA- ITN 2019-2022
Kajangi Gnanachandran

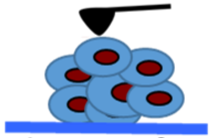
Hypothesis -> larger viscosity of cancer cells, larger cancer invasiveness





Glass surface





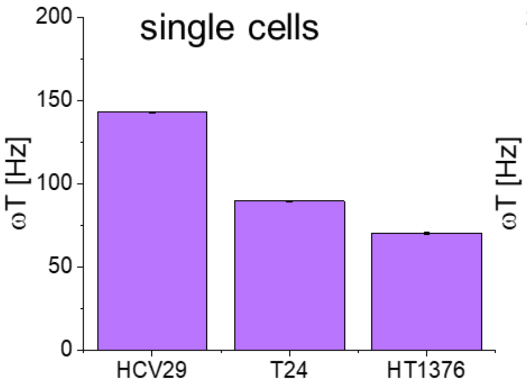
glass surface

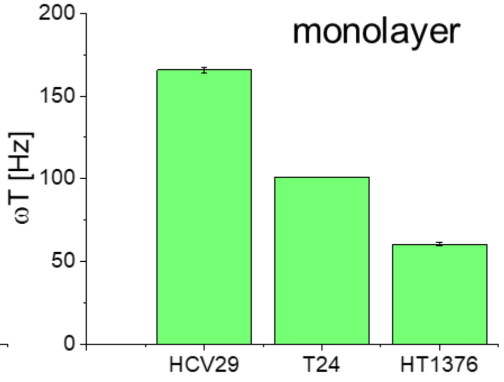
T24 cells are more invasive

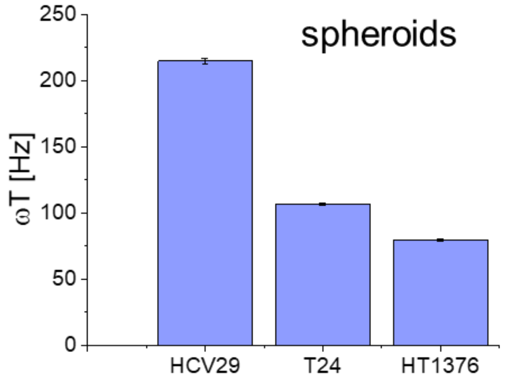
$$G^*(\omega) = \frac{1-\mu}{3\delta_0 tan\theta}\frac{F^*(\omega)}{\delta^*(\omega)}$$

$$G'(\omega) = G_N^0 + k_1\omega^a$$

$$G''(\omega) = k_0\omega^{-n_f} + bk_1\omega^a$$

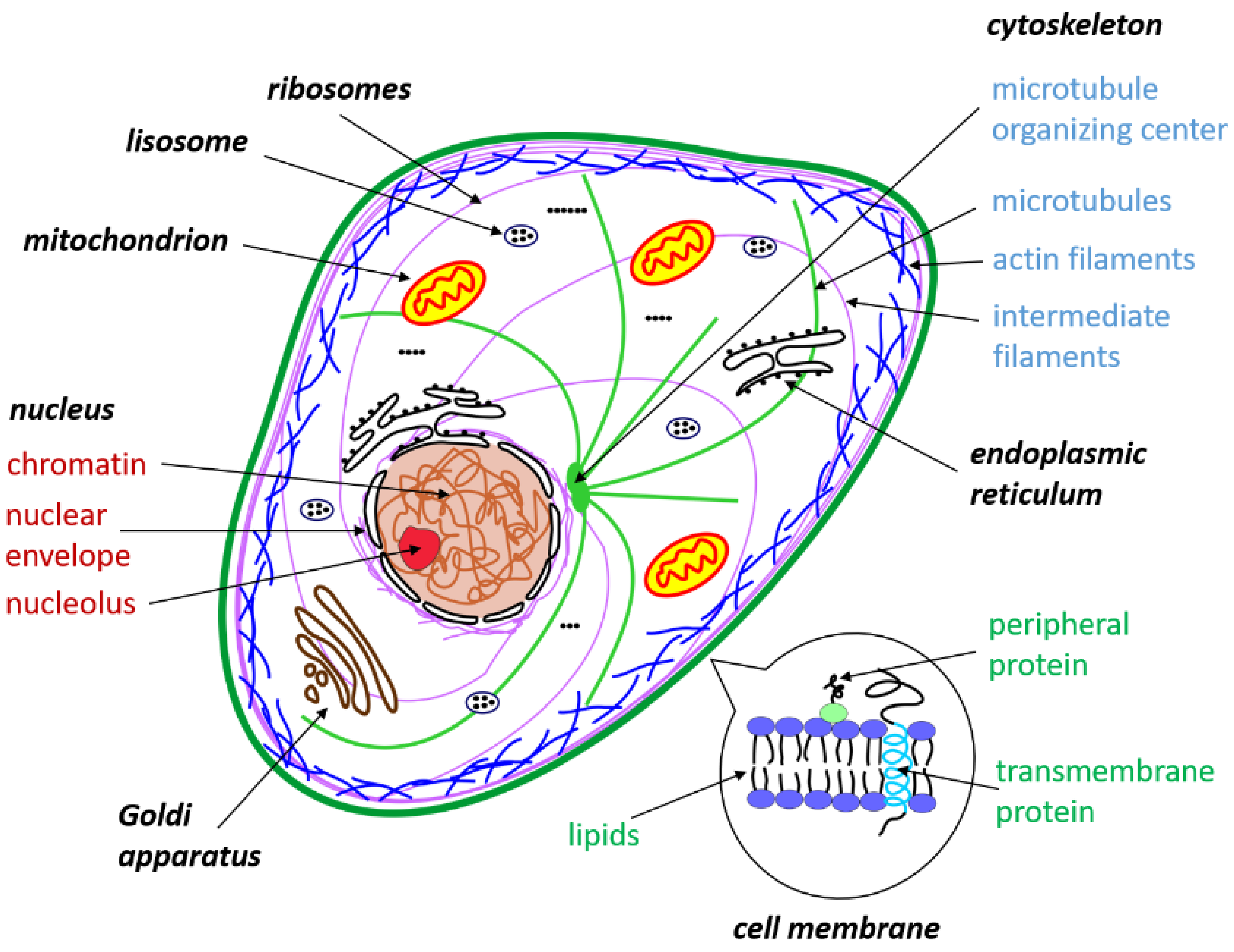






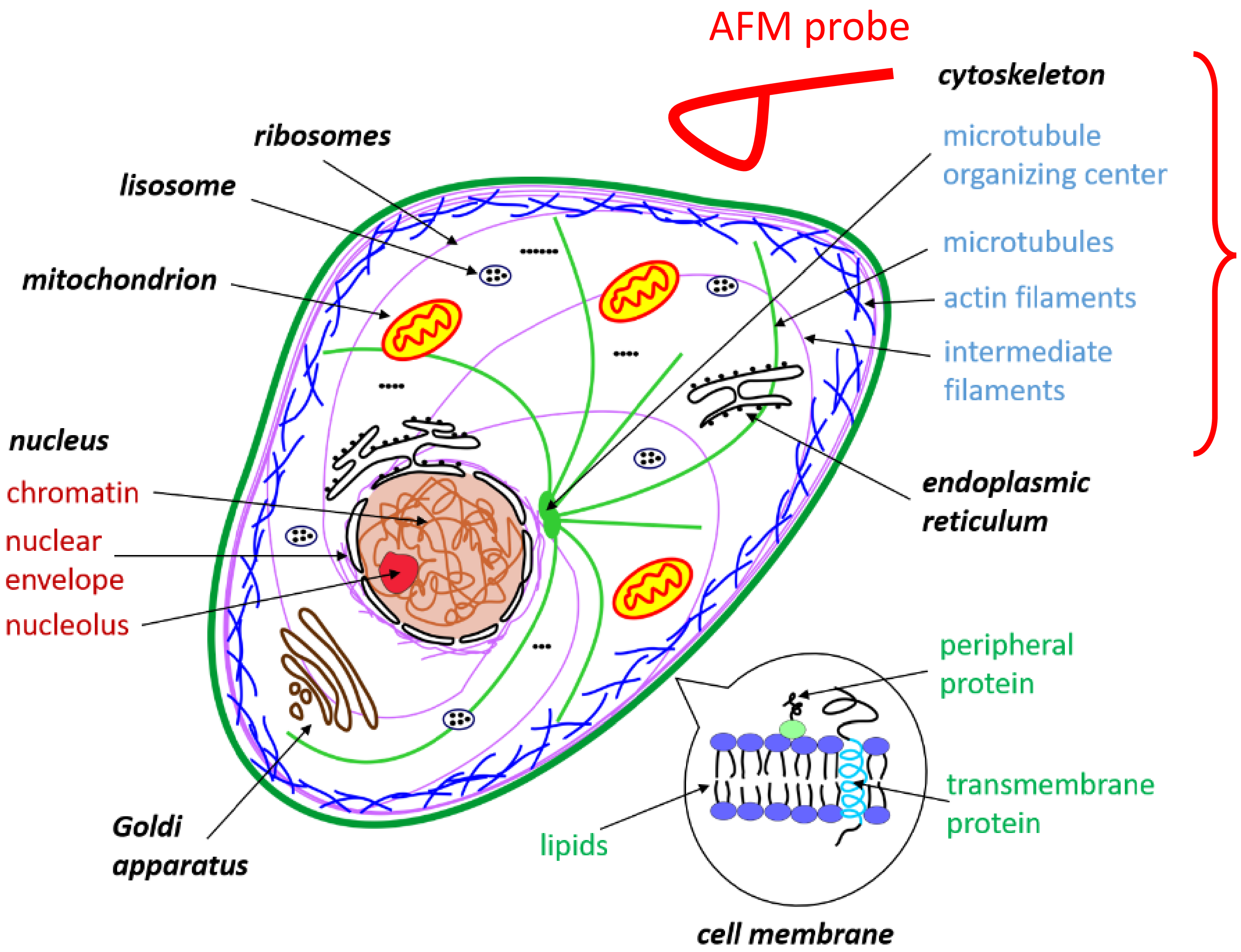
Gnanachandran et al. submitted

Cell cytoskeleton

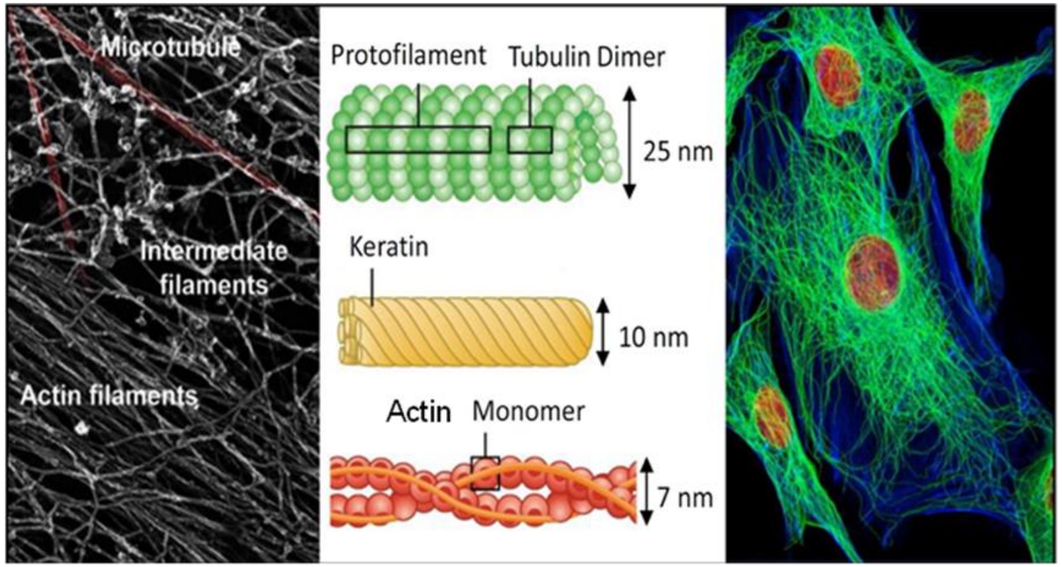


Eds. Lekka, Navaja, Podesta, Radmacher, „Mechanics of diseases", 2022

Cell cytoskeleton



cytoskeletal elements inside the cell

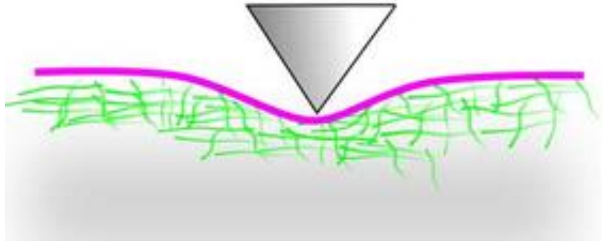


www.olympusmicro.com

Eds. Lekka, Navaja, Podesta, Radmacher, „Mechanics of diseases", 2022

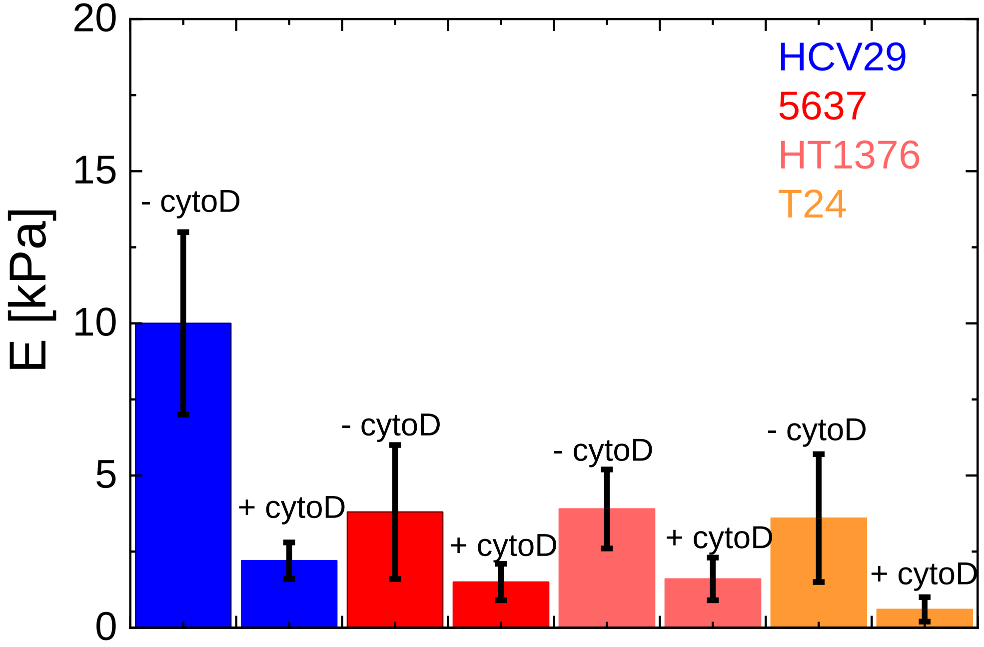
Compressive properties of bladder cancer cells

Are mechanical properties of bladder cancer cells related to actin cytoskeleton?



cytochalasin D (preventing polymerization of F-actin) led to Young's modulus drop

Actin filaments contribute to the mechanical properties of bladder cancer cells

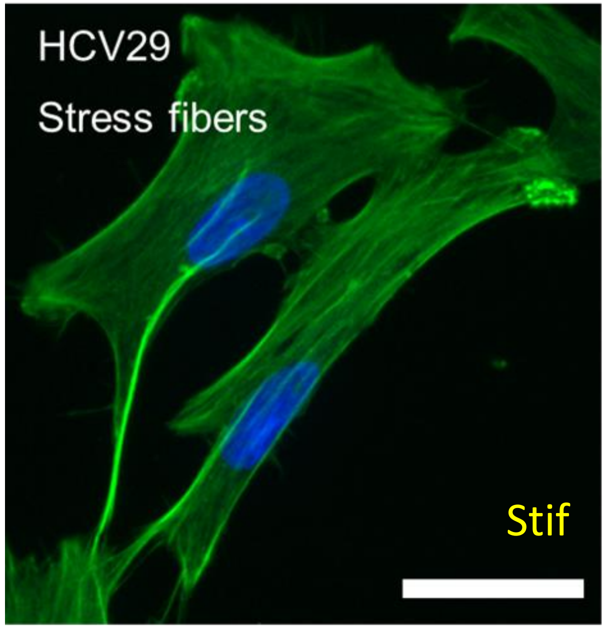


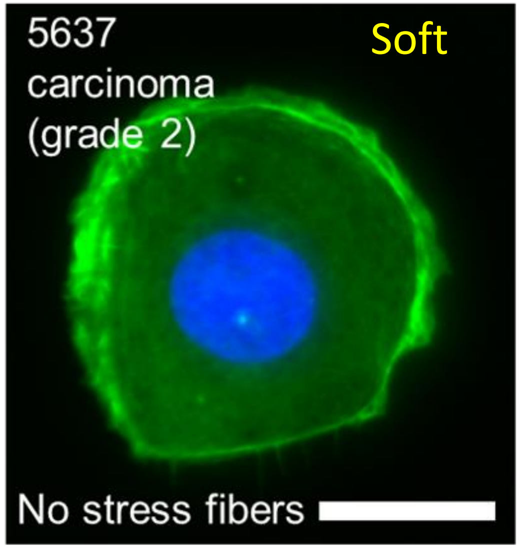
Cytochalasin D treatment (5 μM, 30 min, 37^{o}C)
E was calculated for indentation depth = 500 nm
($p < 0.0001$, for non-treated/treated cells, n = 60 cells)

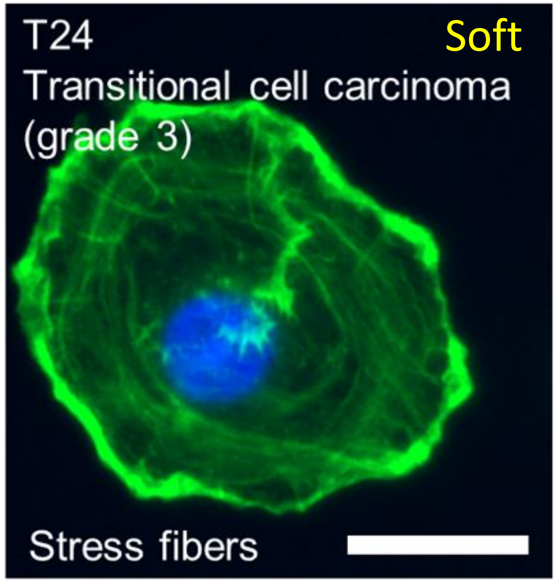
Cell cytoskeleton

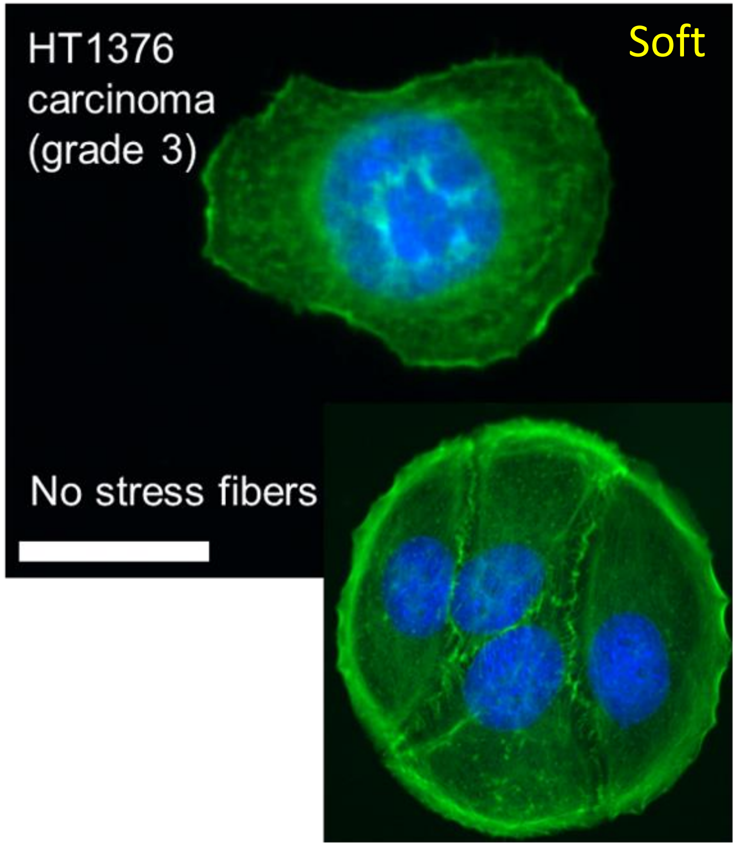
Joanna Pabijan

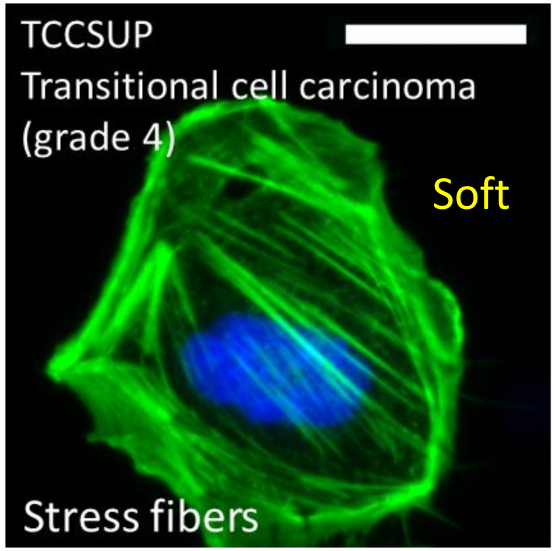
2D organization of actin filaments









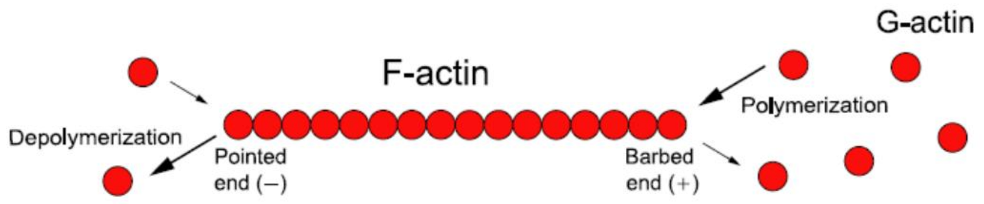


Actin filaments organization weakly contribute to mechanical properties of bladder cancer cells

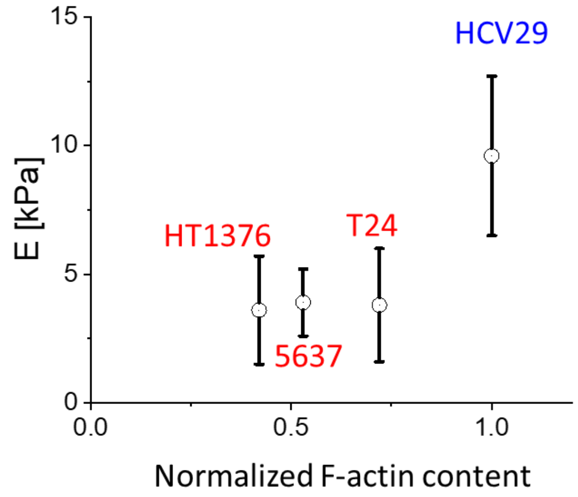
F-actin stained with phalloidin (Alexa-Fluor 488) epifluorescence, scale bar 20 um

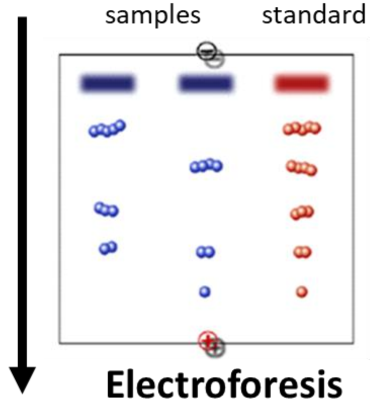
Elastic properties of single cells

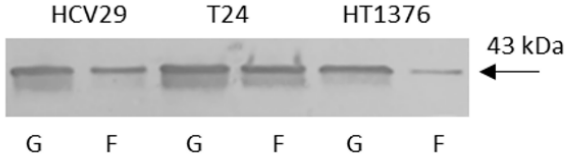
Joanna Pabijan, IFJ PAN,
Sylwia Kędracka-Krok, WBBB UJ
Dorota Gil, CMUJ

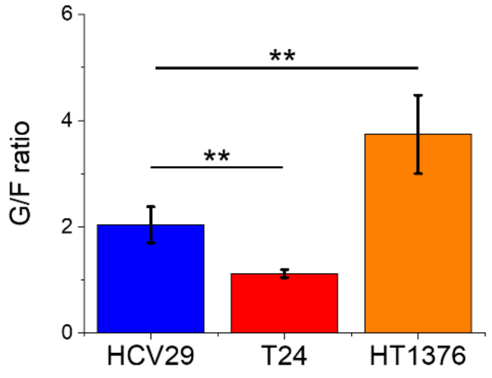


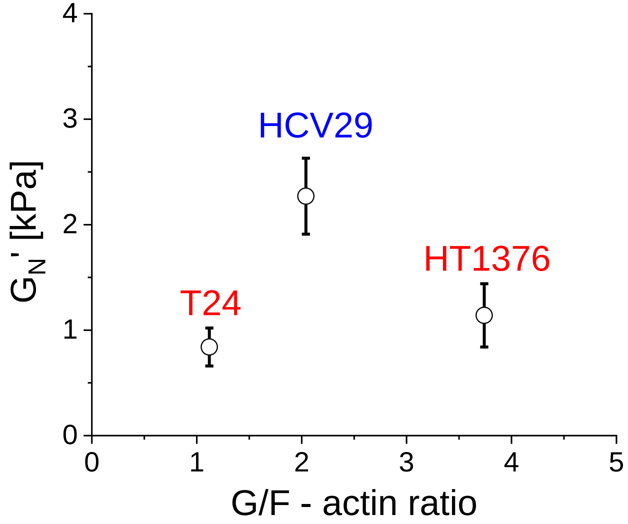
Nonaka et al., Neral Networks, 24 (2011) 979









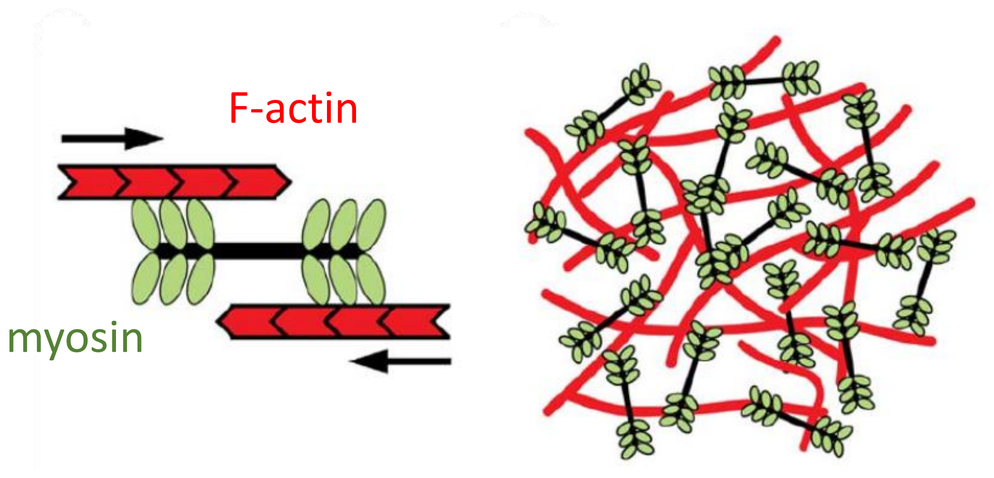


Gnanachandran et al. submitted

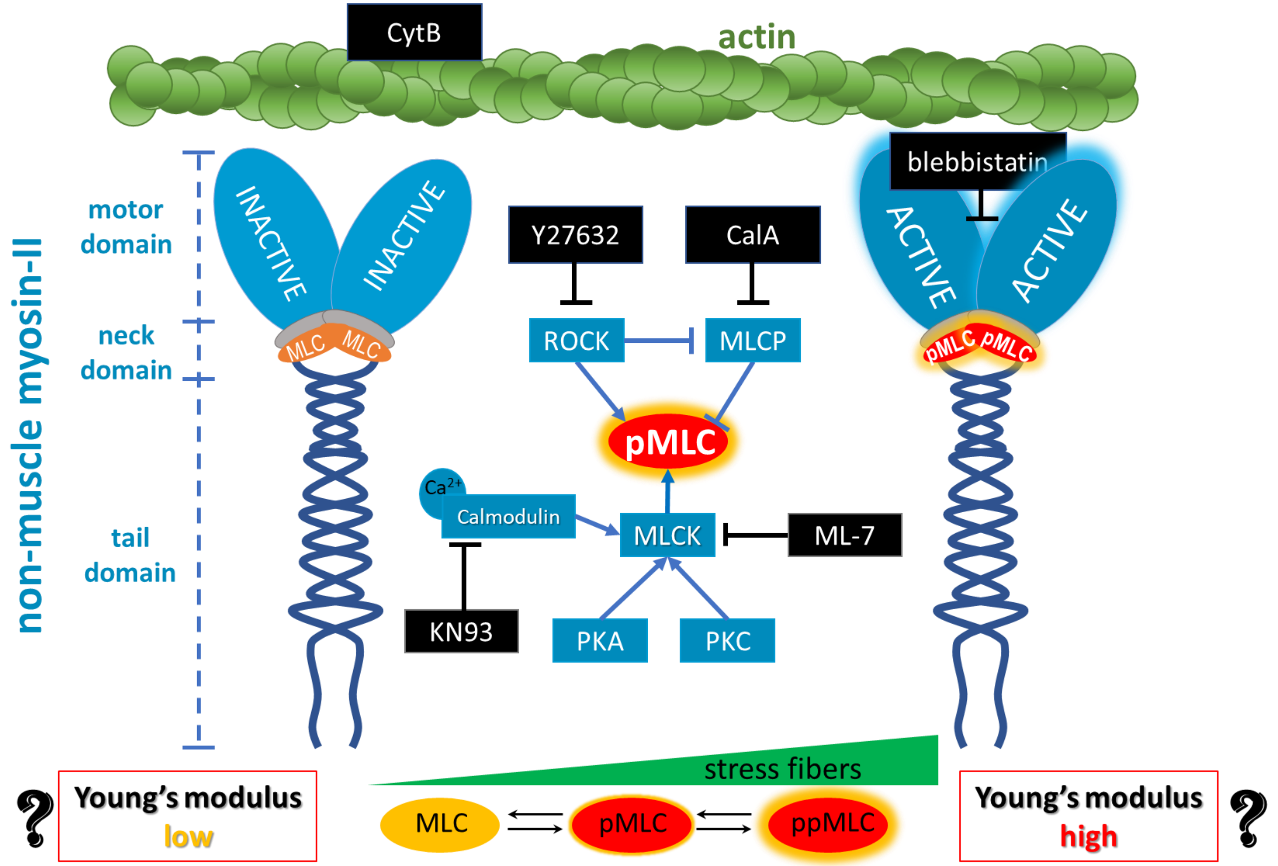
Elastic properties of single cells

AiT(Norway)
Peter McCourt
Bartlomiej Zapotoczny
Tomasz Zieliński
Oliwia Blacharczyk

Does myosin regulate cell Young's modulus?



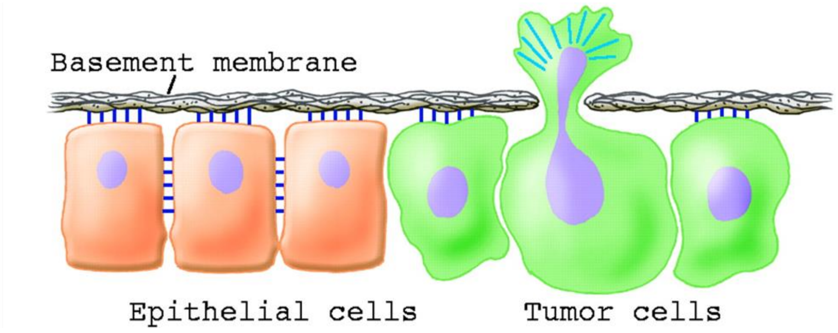
Kasza et al. Curr. Opin. Cell Biol. 23 (2011) 30



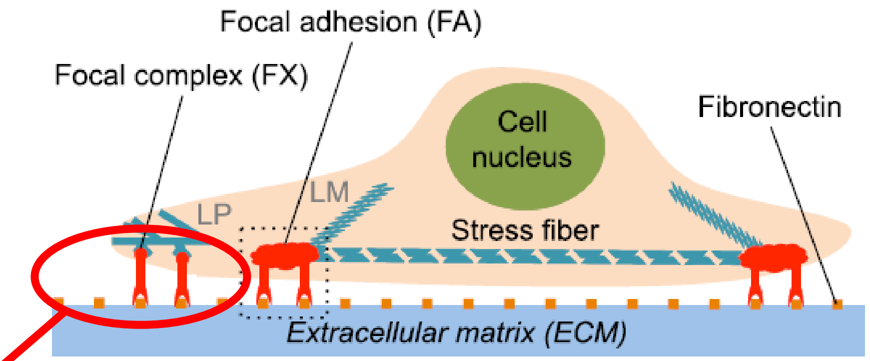
pMLC: phosphorylated myosin (light chain)

Zapotoczny et al. submitted

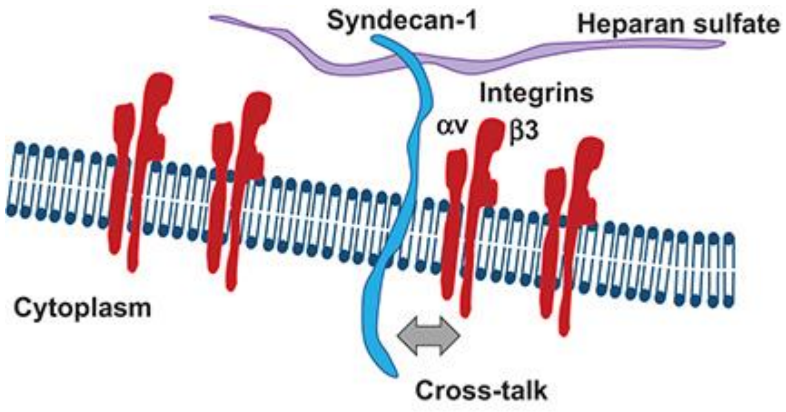
Glycocalix as another component contributing to bladder cancer mechanics



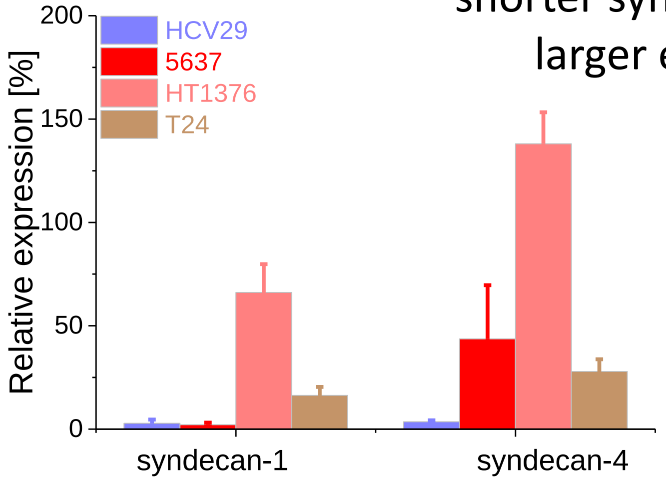
Geho et al. Physiology 20 (2005) 194-200



Hoffmann et al. BMC Systems Biology 7 (2013) 2



Mierke et al. Physics of Cancer, vol. 2 (2018) chap.12

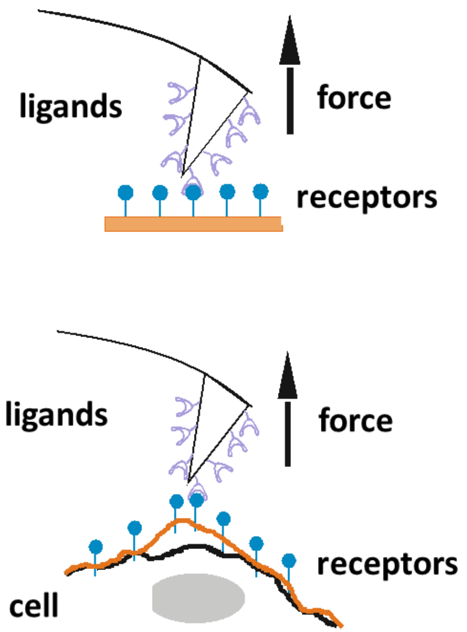


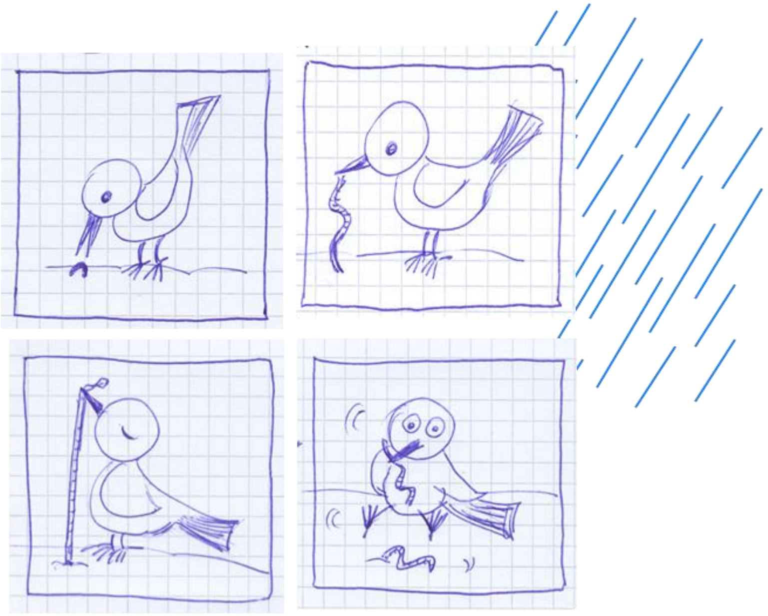
shorter syndecan (SDC-4) larger expression

Atomic force microscopy – interaction forces

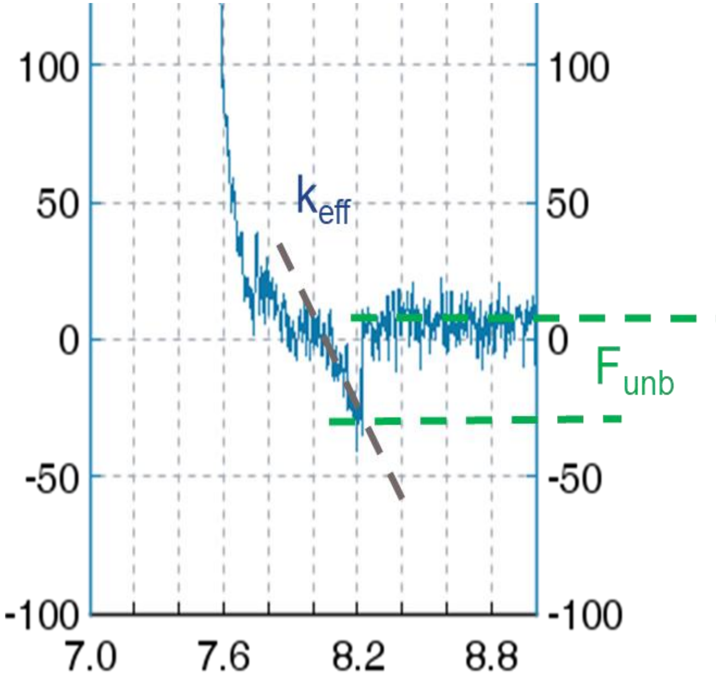
Politechnika Poznańska
Arkadiusz Ptak
Katarzyna Herman
Joanna Zemła

Single Molecule Force Spectroscopy (SMFS)



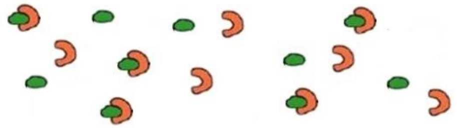


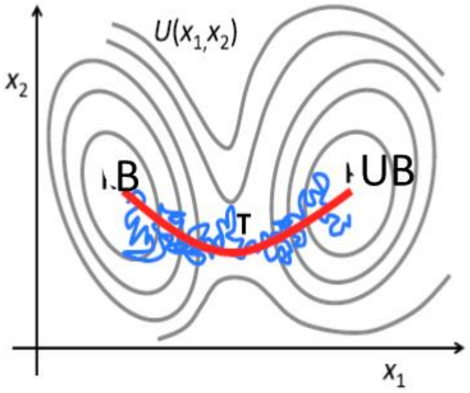
A.Dąbrowska

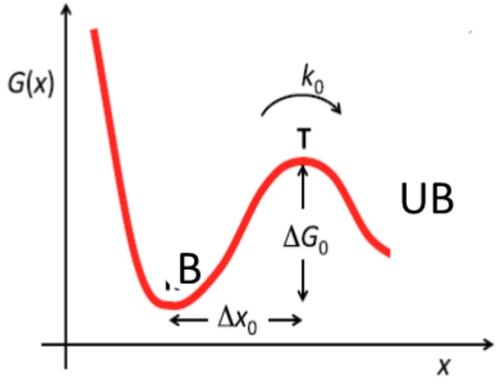


Atomic force microscopy – interaction forces

Dynamic force spectroscopy (DFS)



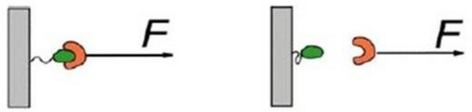


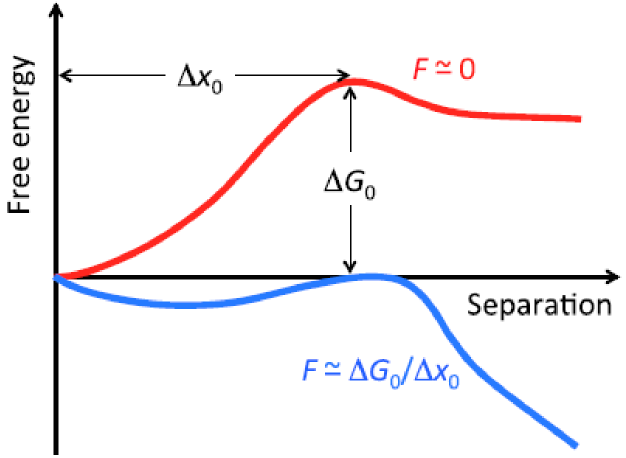


empirical Arrhenius equation

$$k_0 = \mathrm{A} \cdot e^{\frac{-\Delta G_0}{k_b \cdot T}}$$

Arya, Mol. Sim. 2016





Theoretical models:
Bell – Evans
Dudko-Hummer-Szabo
etc.

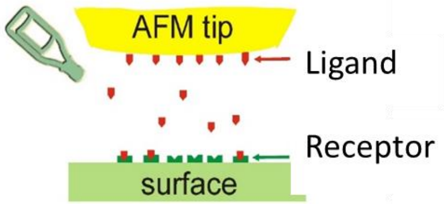
$$F_{unb}(r_f) = \frac{k_b \cdot T}{x_b} \cdot \ln\left(\frac{x_b \cdot r_f}{k_0 \cdot k_b \cdot T}\right)$$

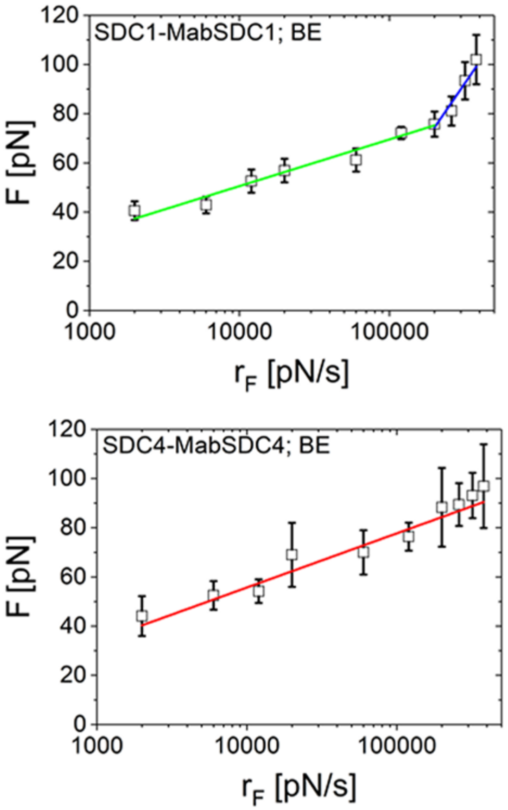
Bell, Science 200 (1978) 618
Evans & Ritchie Biophys. J., 72 (1997) 1541

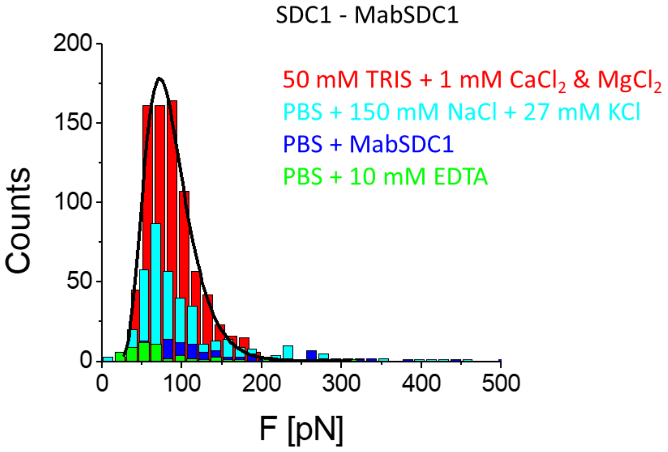
Atomic force microscopy – interaction forces

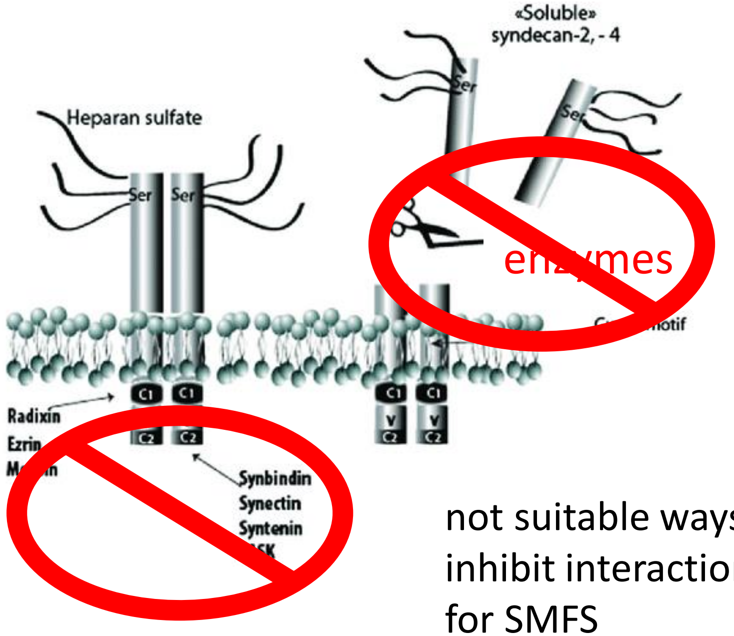
Dynamic force spectroscopy (DFS)

$$F_{unb}(r_f) = \frac{k_b \cdot T}{x_b} \cdot \ln\left(\frac{x_b \cdot r_f}{k_0 \cdot k_b \cdot T}\right)$$









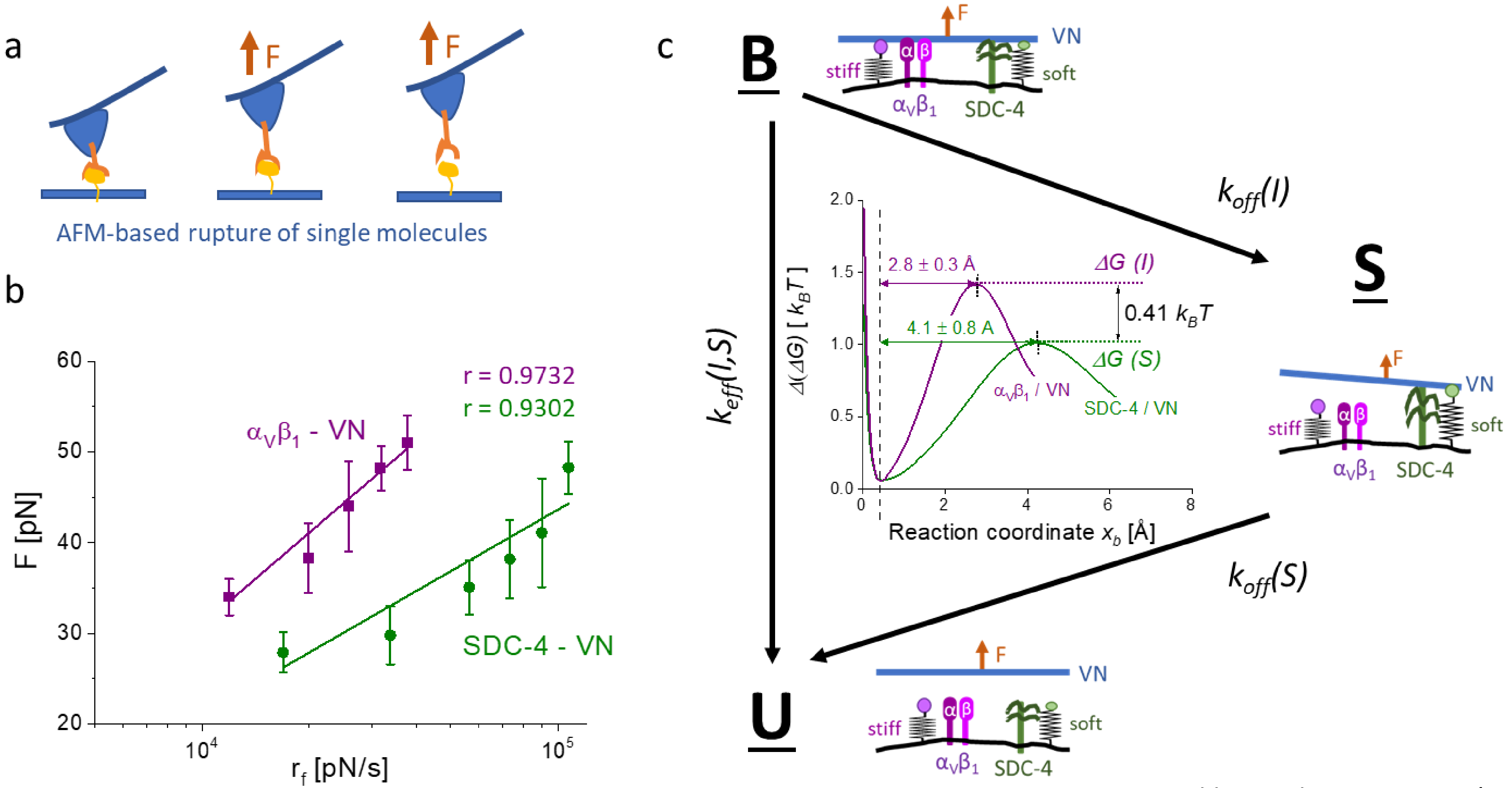
not suitable ways to inhibit interactions for SMFS

Leonova et al., J. Biomol. Struct. Dynam. 33 (2015) 1050

Herman et al. J. Phys. Chem. Lett. 9 (2018) 1509-1515

Atomic force microscopy – interaction forces

Dynamic force spectroscopy (DFS)



Lekka et al. Micron 137 (2020) 102888
Herman et al. Phys. Rev. E 104 (2021) 024409

Summarising results

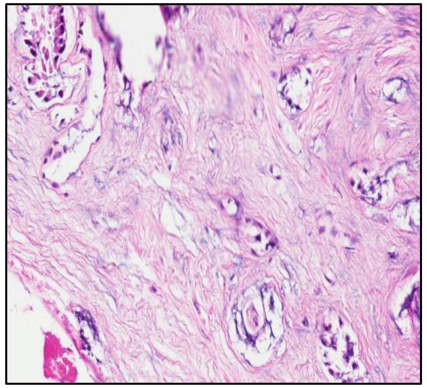
- *Cancer cells are softer - softening of cancer cells already happens at an early stage of cancer progression*
- *Softening of cancer cells is independent of the cell organization (single cells, clusters, monolayers, spheroids, and soft substrates used as a support)*
- *Rheological properties (cellular response to shear forces can be used to classify bladder cancer cells)*
- *F-actin content and G/F ratio (not a 2D organization of actin filaments) contribute to the mechanical properties of bladder cancer cells, but it seems that they are governed by another player (myosin)*
- *Glycocalyx in bladder cells is biphasic, both inner and outer brush are observed but their length and density are different*
- *Syndecans, the most abundant proteoglycans, are overexpressed in bladder cancer (they probably form cell surface brush)*
- *Syndecans are soft springs regulating integrin interaction with proteins outside the cell (extracellular matrix proteins)*

Long-term goals

AIMS:
New classification system for bladder cancers, diagnosis and treatment

Patient qualification

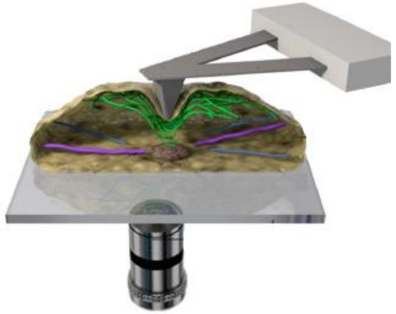
Sample collection



Medical examination

Cells

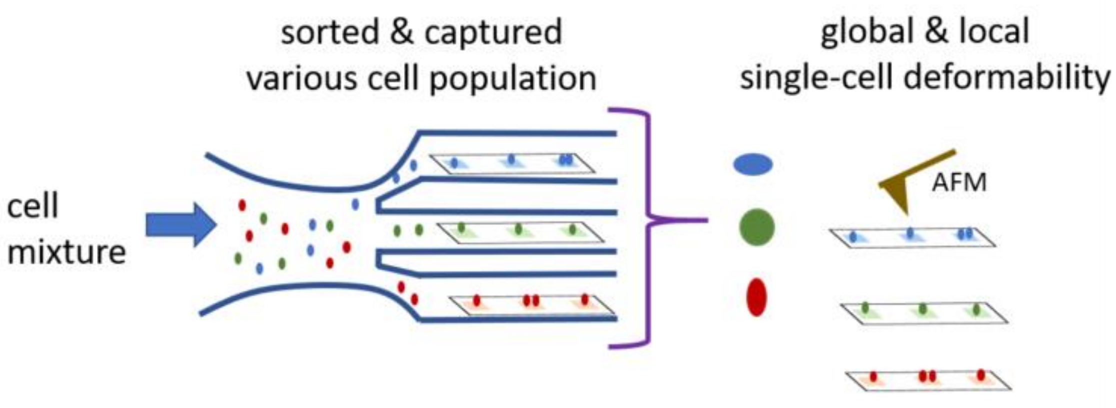
ATOMIC FORCE MICROSCOPY



mechanical, rheological, and adhesive properties of cells

Long-term goal – cell level

Deformability cytometry -> size (shape) deformability, high throughput



AFM -> selected cell population, deformability, adhesion

NTNU

BioMechCanDet

Norwegian Financial Mechanism, Grieg, 2020-2024

IFJ PAN:
ML, J.Zemla, R.Szydlak,
M.Luty, T.Zielinski, J.Pabijan

NTNU:
Bjorn Stokke, Ingrid Haga
Øvreeide, Victorien Prot

Norway grants

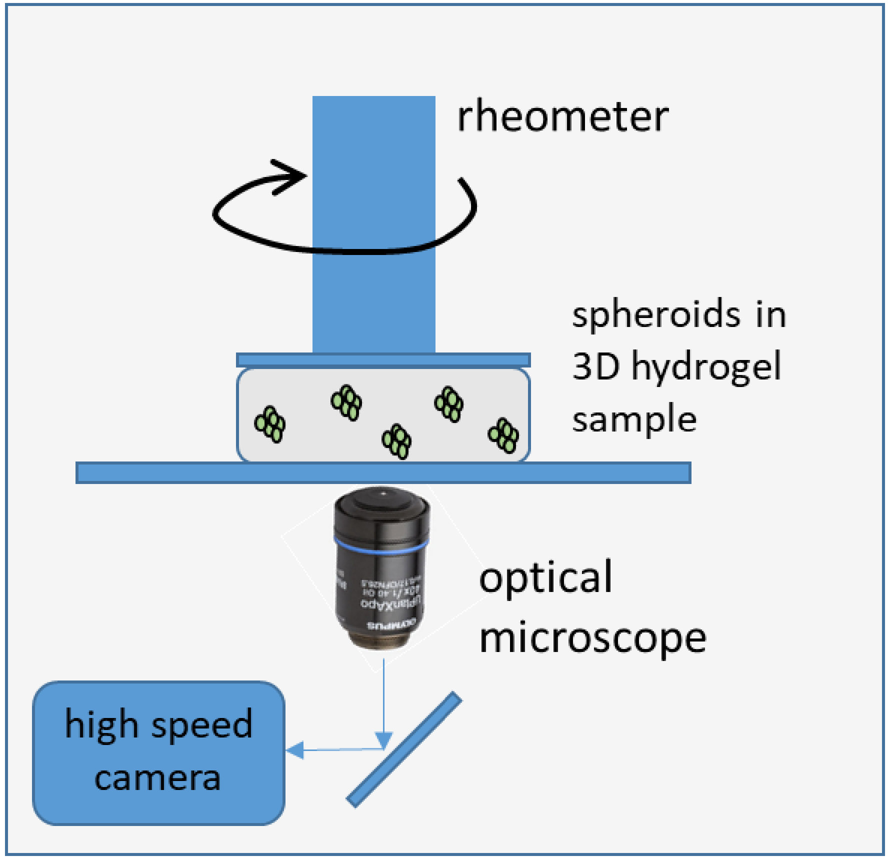
Szydlak et al. in preparation
Luty et al. in preparation
Zemla et al. in preparation

Long-term goal – spheroid/organoid level

NCN, OPUS 2022 – 2026

ML, K.Gnanachandran, J.Pabijan,
post-doc

How shear forces affect cancer cell escape from a tumor

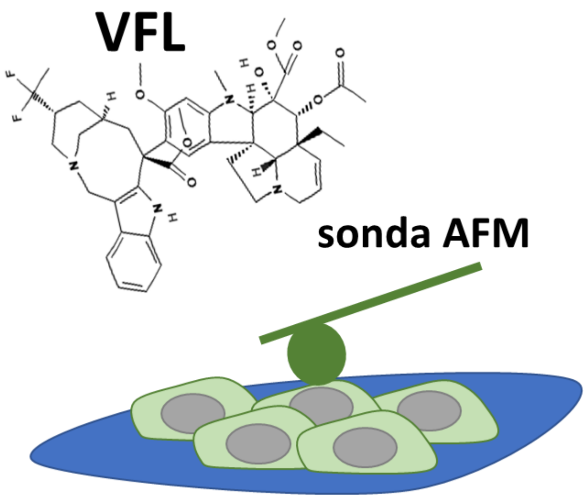


Long-term goal – antitumor drugs

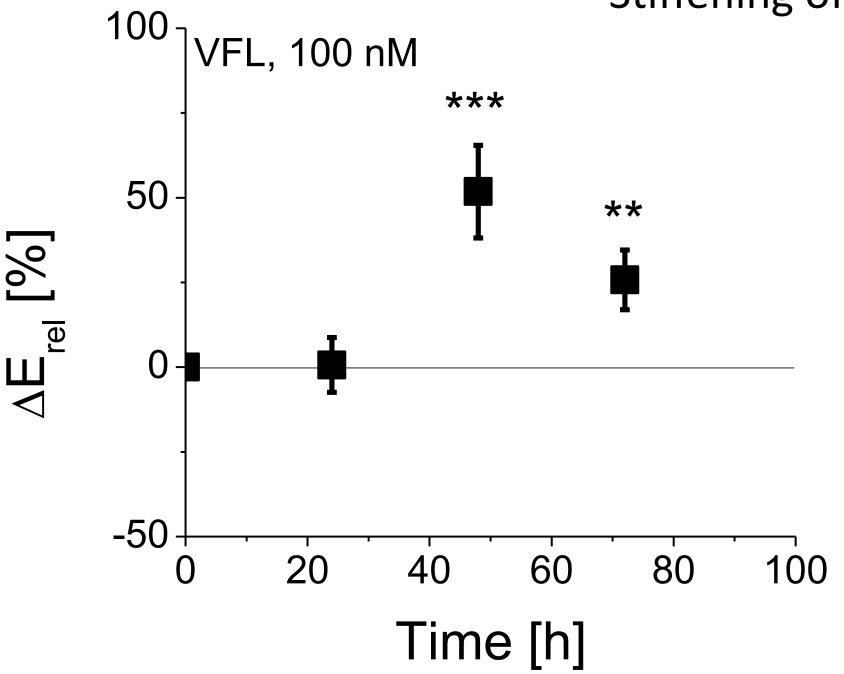
NCN, OPUS 2022 – 2026

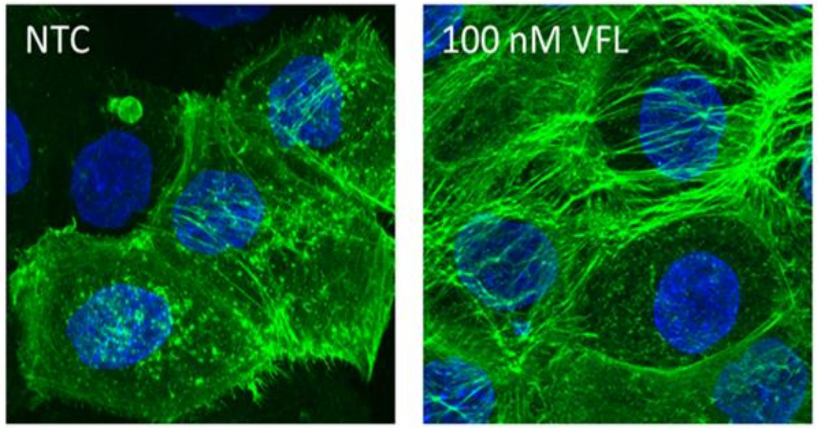
ML, post-doc

how effectively mechanical properties can be combined in pharmacology



Stiffening of cells





Kubiak et al., Nanoscale 13 (2021) 6212

Acknowledgements

Staff:

1. <u>Małgorzata Lekka (head)</u>
2. Marcin Luty
3. Joanna Pabijan
4. Renata Szydlak
5. Bartłomiej Zapotoczny
6. Joanna Zemła

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1. Oliwia Blacharczyk
2. Kajangi Gnanachandran
3. Shruti Kulkarni (shared with U.Bremen)
4. Tomasz Zieliński

http://www.ifj.edu.pl/dept/no5/nz55/

Main collaborations

Tufts University, USA
NTNU, Norway
AiT, Norway
CMUJ, Poland
JCET, Poland

<u>Phys2BioMed beneficiaries</u>

Bremen Univ. (Germany)
Univ. Milano (Italy)
O11 (The Netherlands)
CHUGR - Grenoble (France)

Funding agencies:

Phys2BioMed
no. 812772

Funded by the European Union

2020-2022, no. 812772
EU Horizont 2020, MCSA ITN
Koordynator UMIL, partner IFJ PAN

BioMechCanDet

Norway grants

GRIEG, 2020-2024, UMO-2019/34/H/ST3/00526 (GRIEG), koordynator Małgorzata Lekka
supported by the Norwegian Financial Mechanism for 2014–2024

NATIONAL SCIENCE CENTRE
POLAND

- OPUS, 2022-2026, UMO-2021/43/B/NZ4/01133, koordynator IF PAN, partner IFJ PAN
- OPUS 2022-2026, UMO-2021/41/B/ST5/03032, kierownik Małgorzata Lekka
- SONATA 2020-2023, UMO-2019/35/D/NZ3/01804, kierownik Bartłomiej Zapotoczny
- Submitted OPUS 23 -> kierownik Joanna Zemła


Thank you