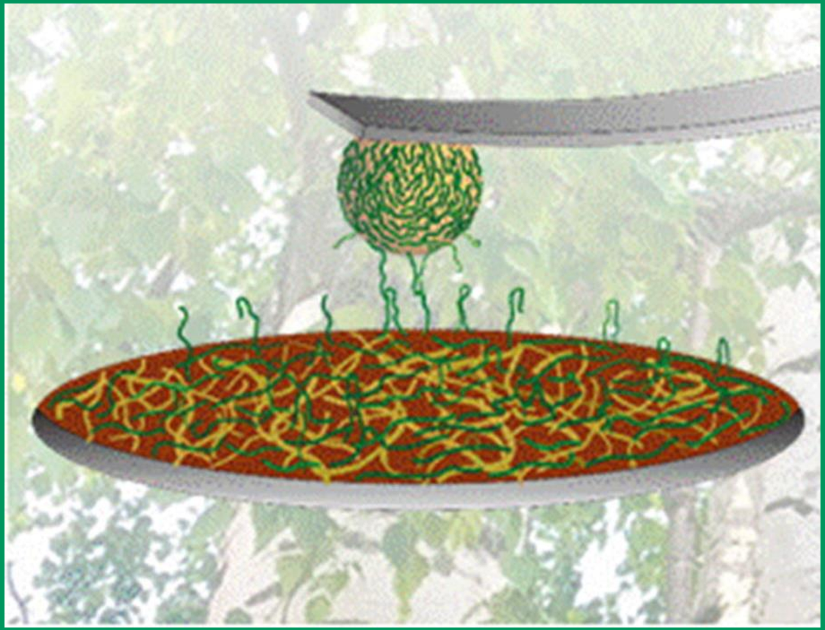
CHEM-L2000 Ultrathin films

Atomic force microscopy II: Colloidal Probe



Monika Österberg

26.2.2024

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Engineering

Learning outcomes

After this lecture you

- **Understand why is it useful to measure direct surface forces and friction forces**
- **Know the main requirements to get reliable results**
- **You are familiar with various force measuring strategies**
    - Ranging from chemical force spectroscopy to single cell force spectroscopy – focus on colloidal probe microscopy

Why measure forces?

Have you measured surface forces using AFM?
Have you measured interactions in any other way?
Are surface forces relevant in your research?

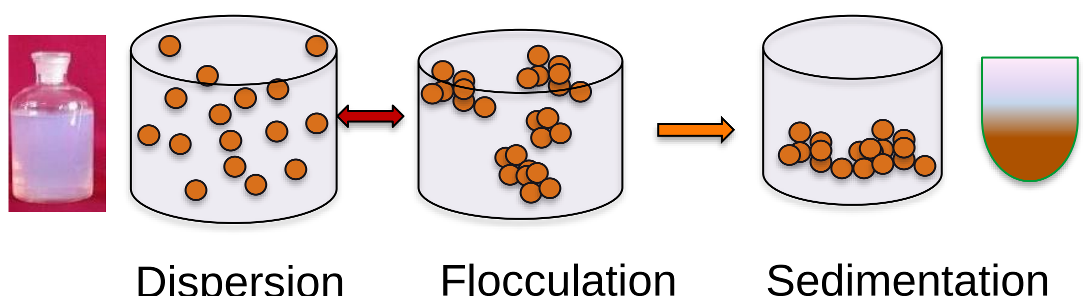
Nano vs. colloidal vs. macroscopic

- In nanoparticle dispersions the surface forces between particles determine if the particles aggregate or stay well dispersed

We can predict (and affect) flocculation and stability of dispersions.

Our knowledge of interaction of e.g. charged, hydrophobic or polymer coated particles in aqueous solutions are based on careful surface force measurements.

For more complex systems measurements are needed to increase our understanding.



The basic principle of force measurements

The base of a spring is moved by known amount ΔD_0.
Due to forces between the surfaces the spring deflects ΔD_s while the surface separation changes by ΔD.

$$\Delta D_s = \Delta D_0 - \Delta D$$

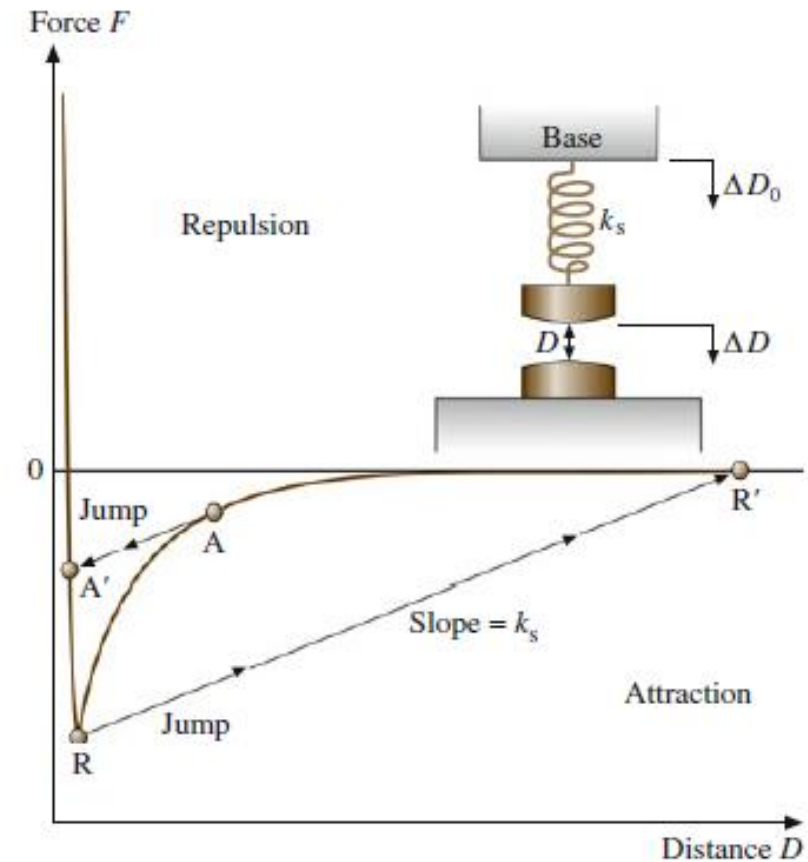
The difference in force, between the initial and final separation is given by

$$\Delta F = k_s \Delta D_s$$

Where k_s is the spring constant

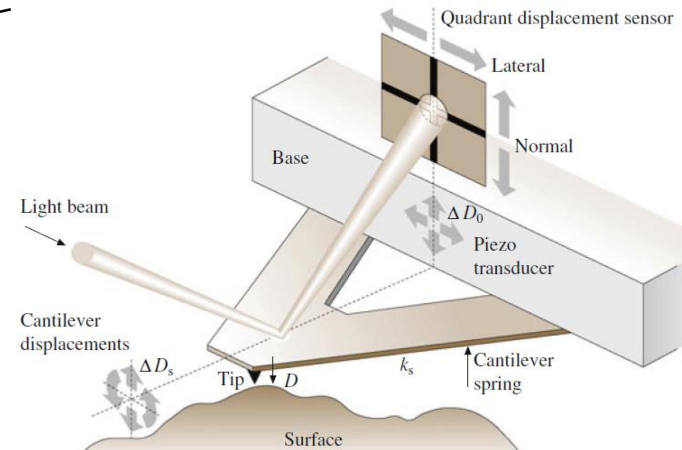
By measuring ΔF at various distances from zero force to hard wall contact the whole force curve (law) can be constructed.

The force law *F(D)*



Ruths & Israellachvili in Nanotribology and Nanomechanics II Bhushan (Ed), Springer, 2011, Berlin, pp107.

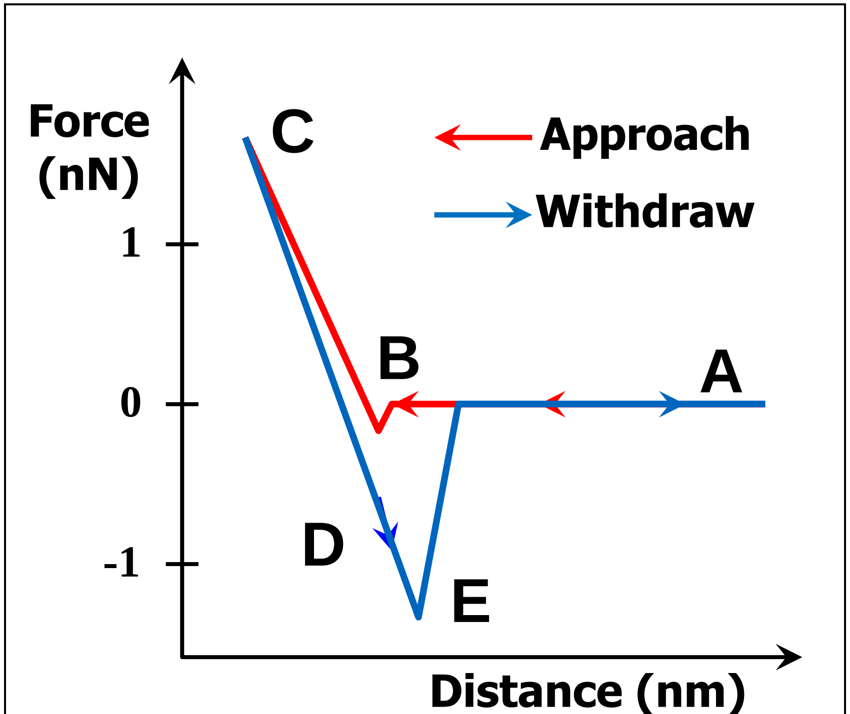
Atomic force microscopy

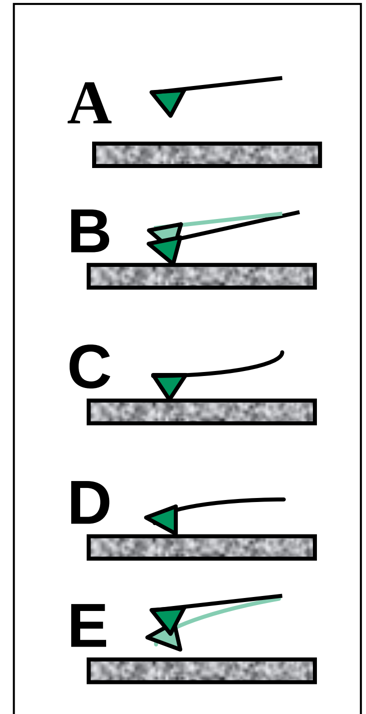


Ruths & Israellachvili in Nanotribology and Nanomechanics II Bhushan (Ed), Springer, 2011, Berlin, pp107.

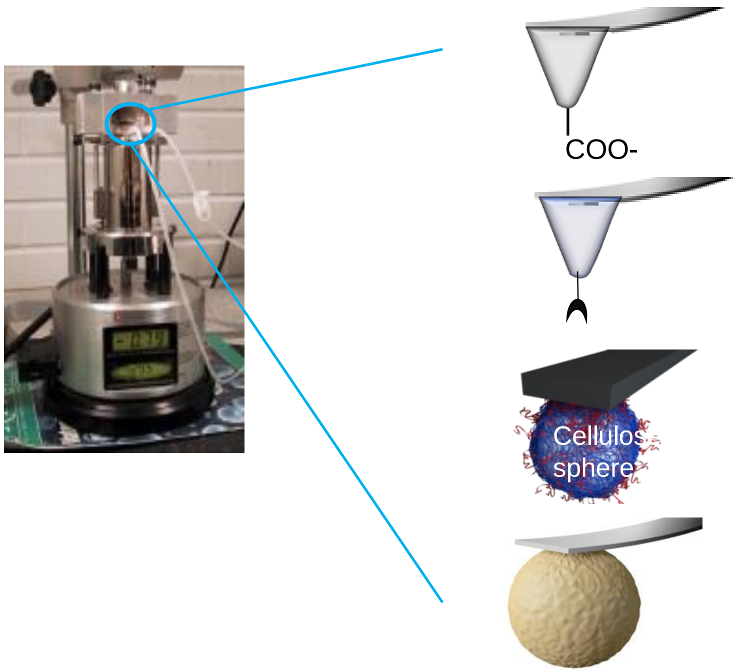
Not just imaging – Also direct quantification of forces between surfaces

Principle of force spectroscopy





Direct surface force measurements – Information on specific and non-specific interactions



Chemical force microscopy (CFM)

Single-molecule force spectroscopy (SMFS)

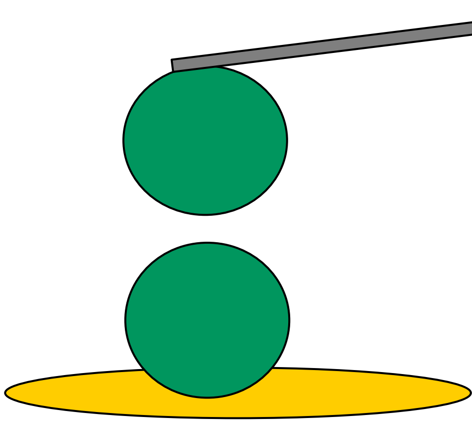
Colloidal probe microscopy (CPM)

Single-cell force spectroscopy (SCFS)

Colloidal probe microscopy

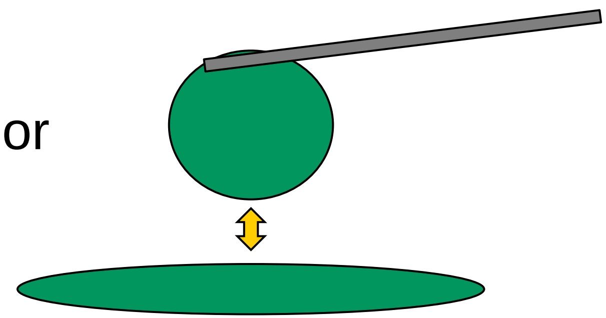
Well-defined geometry facilitates correlation to theory

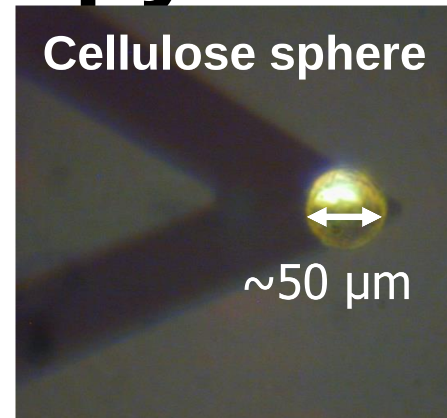
Sphere against sphere

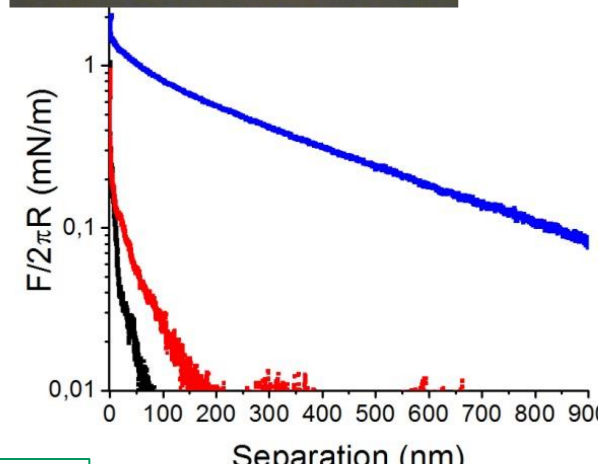


or

Sphere against plane



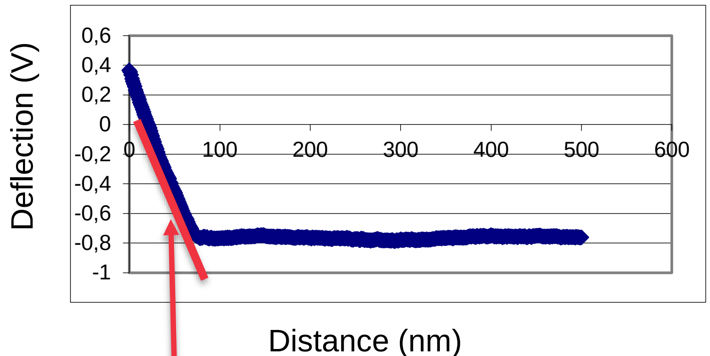




Very simplified: From the shape of the force curve we can say what forces are important in the system: electrostatic, steric, hydrophobic,…

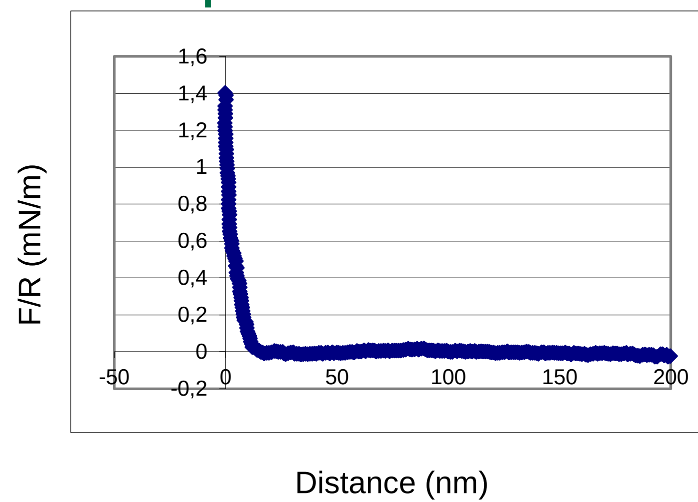
AFM force spectroscopy in practice

Raw data = deflection as a function of relative distance



How do you get from raw data to force as a function of distance between probe and substrate?

Force (normalized with the radius of the sphere) as a function of distance between probe and substrate



Sensitivity, spring constant, radius of sphere(s) has to be determined

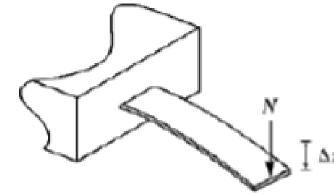
For soft materials the sensitivity has to be determined on a hard substrate

Determination of normal spring constant

$$spring\ constant\ k_z = \frac{N}{\Delta z}$$

Force/deflection

$$(k_s = \frac{F}{\Delta D})$$

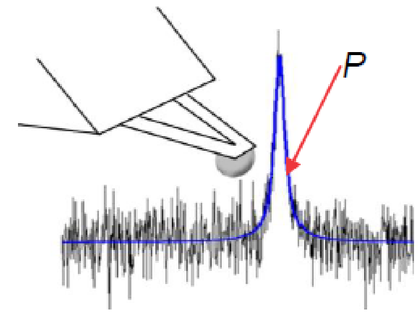


The thermal noise method

Measures the resonance frequency peak determined from the analysis of the thermal fluctuations of the cantilevers

$$spring\ constant\ k_z = \frac{kT}{P}$$

k= Boltzmann's constant, T = Temperature, P area of the power spectrum of thermal fluctuation



https://doi.org/10.1016/j.colsurfa.2013.11.018

The Sader method

In addition to the thermal fluctuation and sensitivity, the dimensions of the cantilever needs to be known

$$k_z = 0.1906\rho b^2 L Q_f \omega_f^2 \Gamma_i^f(\omega_f)$$

ρ is the density of the fluid, *b* and *L* are the width and length of the cantilever, respectively, Qf is the quality factor and Γ_f is the imaginary component

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Green et al Review of Scientific Instruments 75, 1988 (2004); https://doi.org/10.1063/1.1753100

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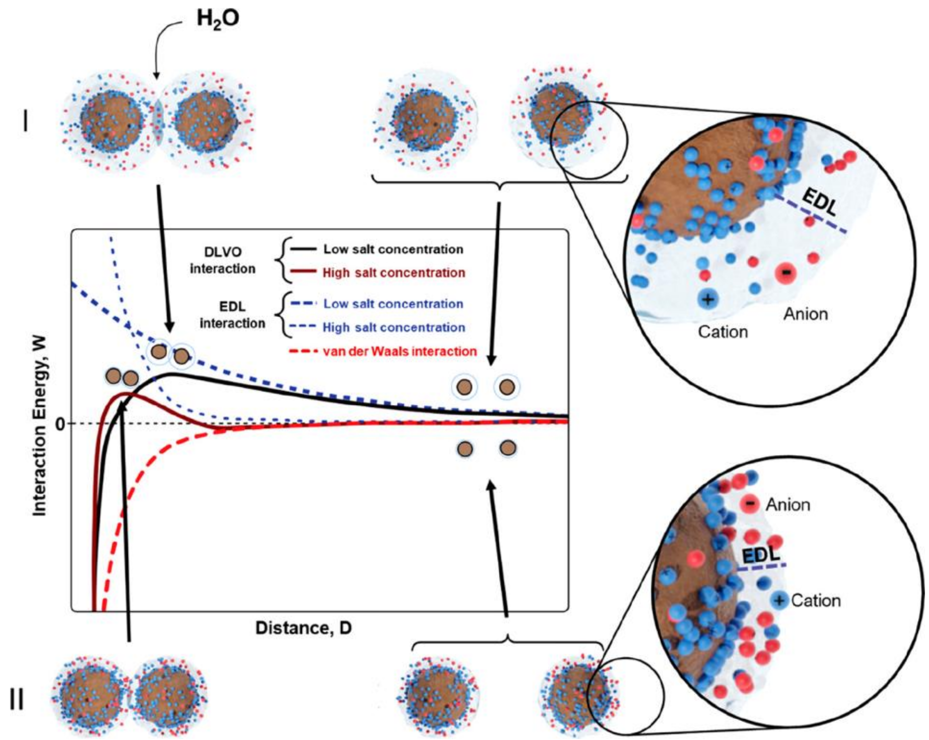
Surface forces: A *very* simplified overview

Electrical double layer (EDL) repulsion and van der Waals attraction



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Österberg, M., Henn, K.A., Farooq, M. and Valle-Delgado, J.J., 2023. Biobased Nanomaterials— The Role of Interfacial Interactions for Advanced Materials. *Chemical reviews*, *123*(5), pp.2200-2241.

Electrical double layer repulsion between like charged particles

Around charged particles there is a diffuse layer of small ions leading to net zero charge of the particle + ions. Hence the concentration of counterions in the diffuse layer is higher than the concentration of co-ions.

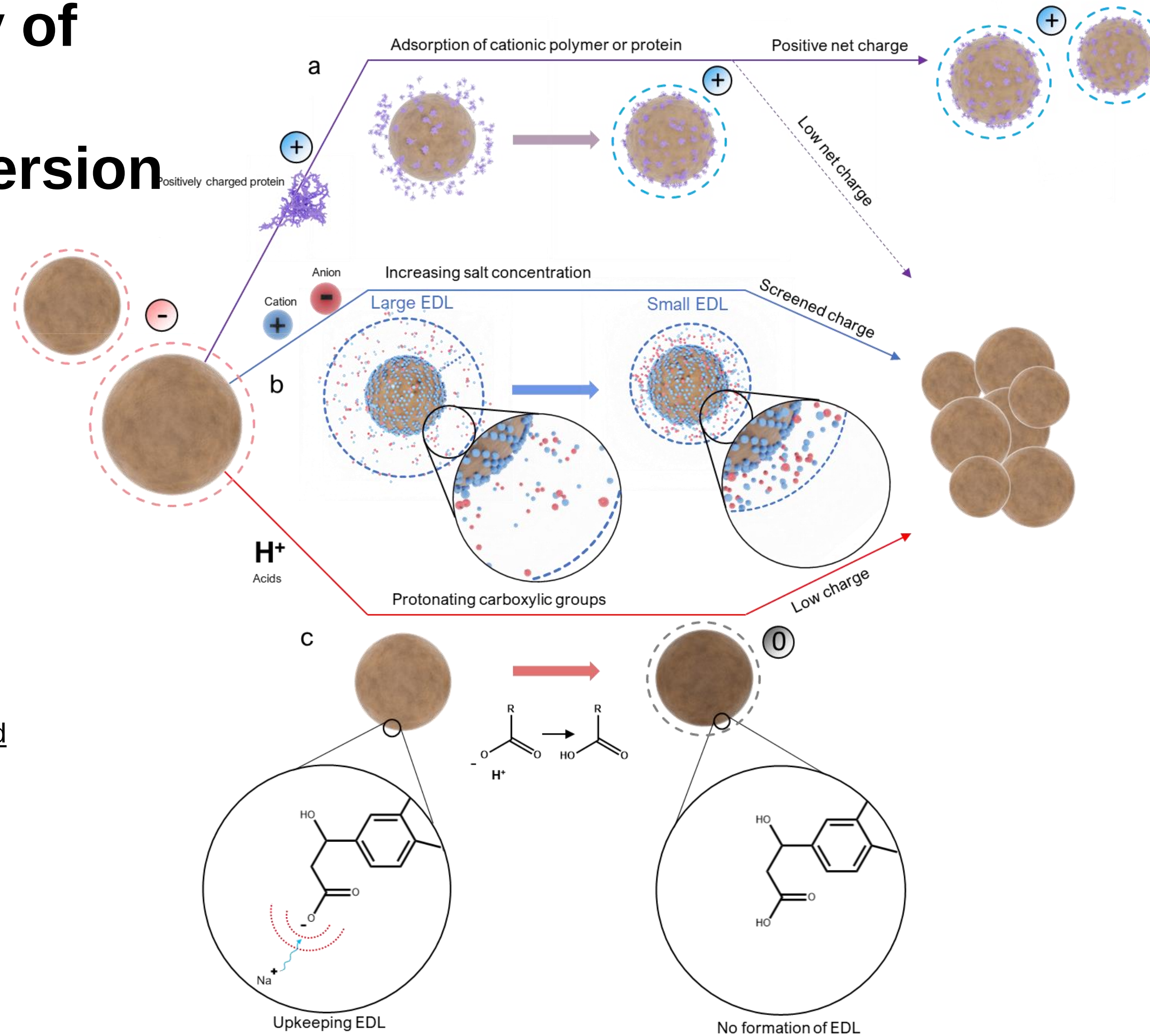
The thickness of the EDL depends on the salt concentration.

I) At low salt concentration the EDL is thicker. As a consequence, the EDL repulsion starts at larger distances between particles leading to more efficient repulsion and stable dispersion.

II) In high salt concentration the EDL is thinner, and particles can come closer. This enables attractive van der Waals forces to dominate leading to attractive forces and aggregation.

Note: The magnitude of the EDL repulsion depends on the charge of the particles while the distance of the repulsion depends on salt concentration.

Example: Stability of aqueous lignin nanoparticle dispersion



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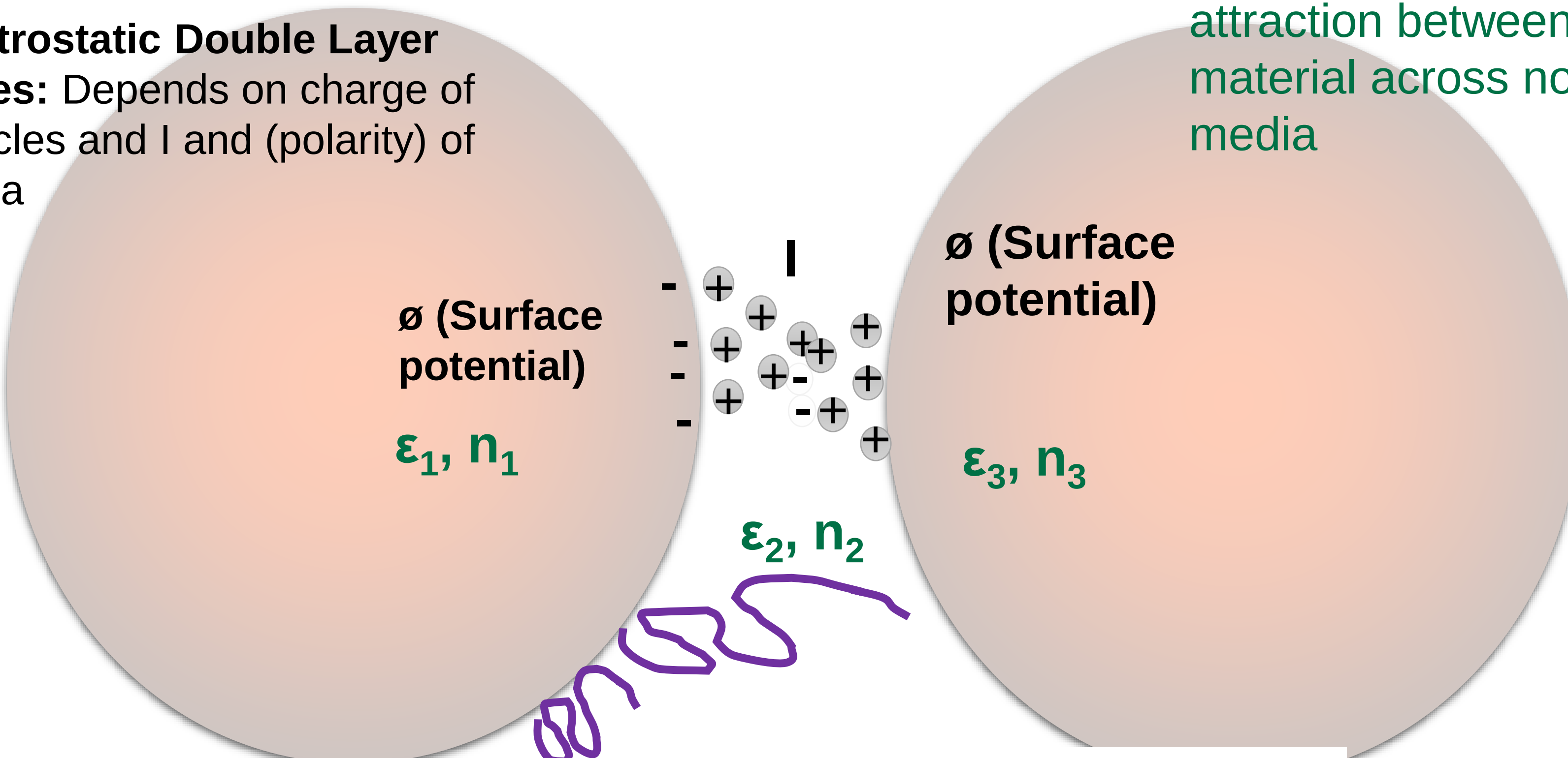
Surface forces mind map

vdW forces: Depends on polarity of particles and media. Highest attraction between polar material across nonpolar media

Electrostatic Double Layer forces: Depends on charge of particles and I and (polarity) of media



Polymers: Attractive bridging force or steric repulsion. Depends on coverage and interactions between polymer and solvent.

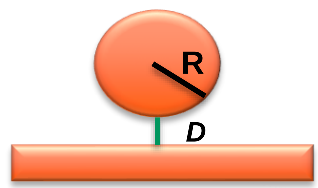
DLVO theory - summary

DLVO-theory

- Derjaguin, Landau, Verwey and Overbeek
- $F_{tot} = F_{van\ der\ Waals} + F_{electrostatic}$ (F = force)

A_H = Hamaker constant, a measure of the "polarity difference" between particles and media

$F_{vdW} = -A_H R/12D^2$



$$F_{DL} \approx 2\pi\varepsilon_0\varepsilon_r R\kappa\phi^2 \exp(-\kappa D)$$

Debye length, $\kappa^{-1} = \sqrt{\frac{\varepsilon_0\varepsilon_r kT}{2e^2 I N_A}}$

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Can you give examples of systems that can be described by DLVO theory?

R= radius of sphere

D = distance between surfaces

k= Boltzmann constant

T = temperature

ε = static dielectric constants for the three media

A_H = Hamaker constant for the system

ε_0 = vacuum permittivity

Ø =Surface potential

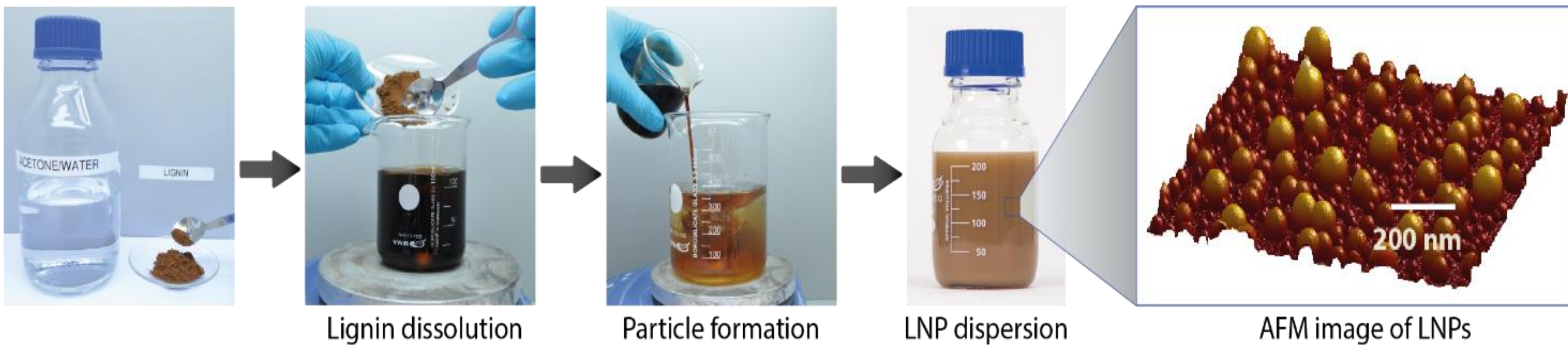
N_A = Avogadros constant

I = ionic strength $I = \frac{1}{2}\sum_i z_i^2 c_{0,i}$

z = valency of ion

C_0 = concentration of ion i

Colloidal lignin particle (CLP) preparation via nanoprecipitation



Produces ***stable*** aqueous dispersion of ***spherical*** lignin nanoparticles (diameter~100 nm)

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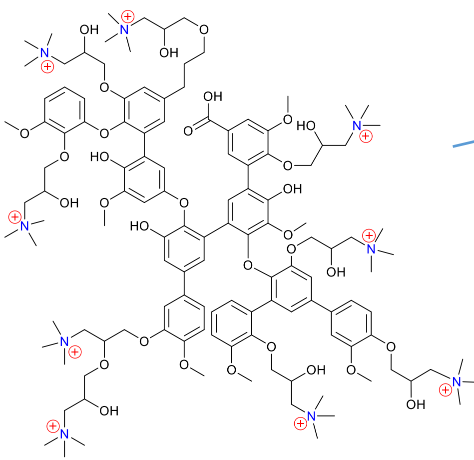
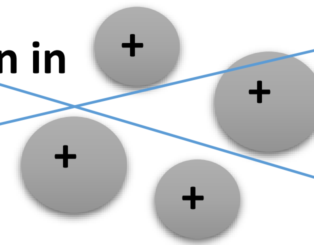
How to make cationic lignin particles?



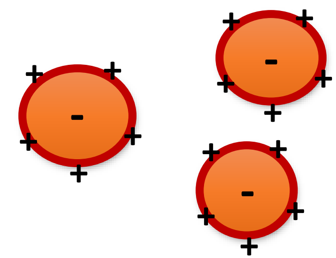
Cationic lignin

Reaction with glycidyltrimethylammonium chloride

~~**Direct precipitation in antisolvent**~~



Adsorption to anionic colloidal lignin particles



- **Stable**
- **High cationic charge possible**
- **Minimum amount of nonrenewable chemicals**

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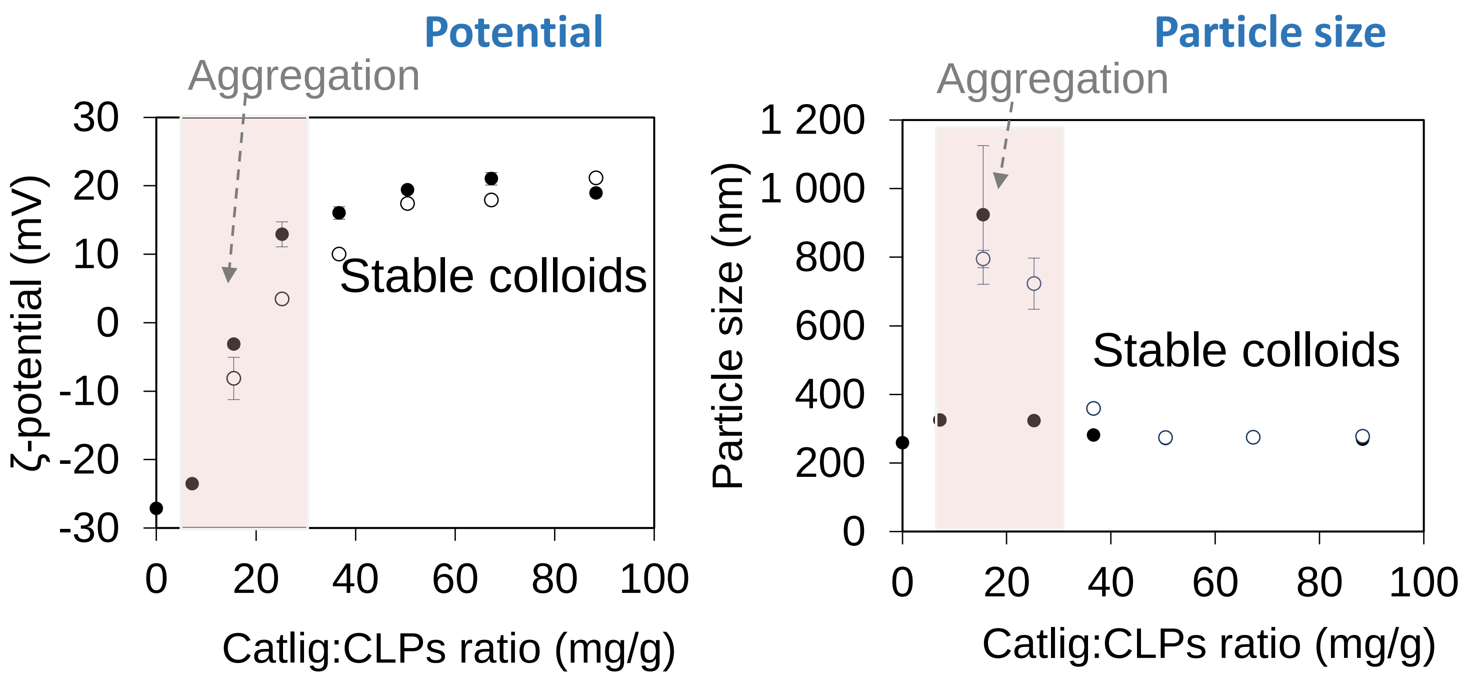
Dispersion stability of cationic lignin particles



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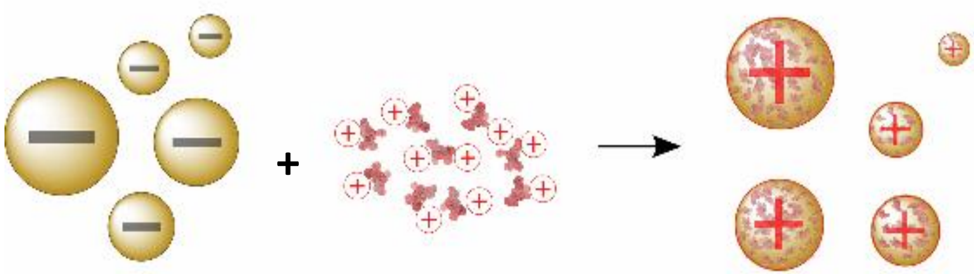
Explanation

Low surface coverage → Charge neutralization



High surface coverage → Stable cationic particles, Overcompensation

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Derjaguin approximation

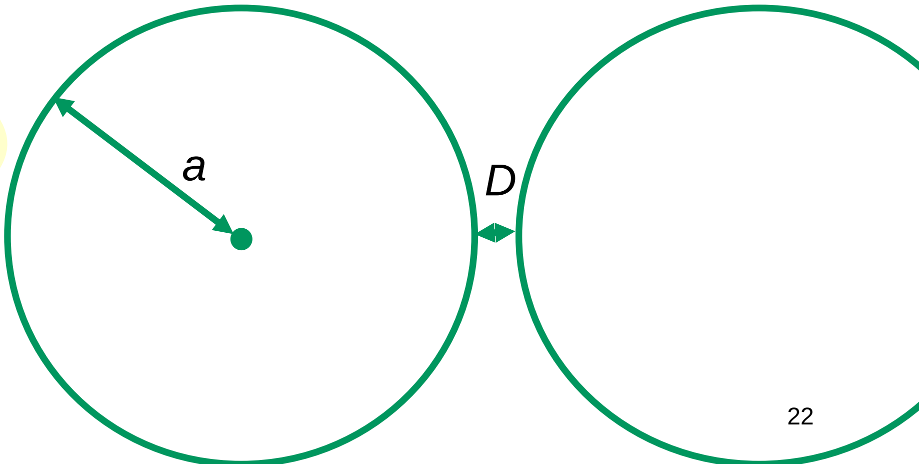
Why do we normalize with the radius of the sphere(s)?

The interaction energy between two flat surfaces =

$$W(D) = \frac{F_{spheres}(D)}{2\pi\left(\frac{a_1 a_2}{a_1 + a_2}\right)} = \frac{F_{cylinders}(D)\sin\theta}{2\pi\sqrt{a_1 a_2}} = \frac{F_{sphere+flat.surf}(D)}{2\pi}$$

•Valid if *D (distance between the surfaces)* << *a (radius)*

Normalisation makes it possible to compare measurements



Examples of measured forces using AFM

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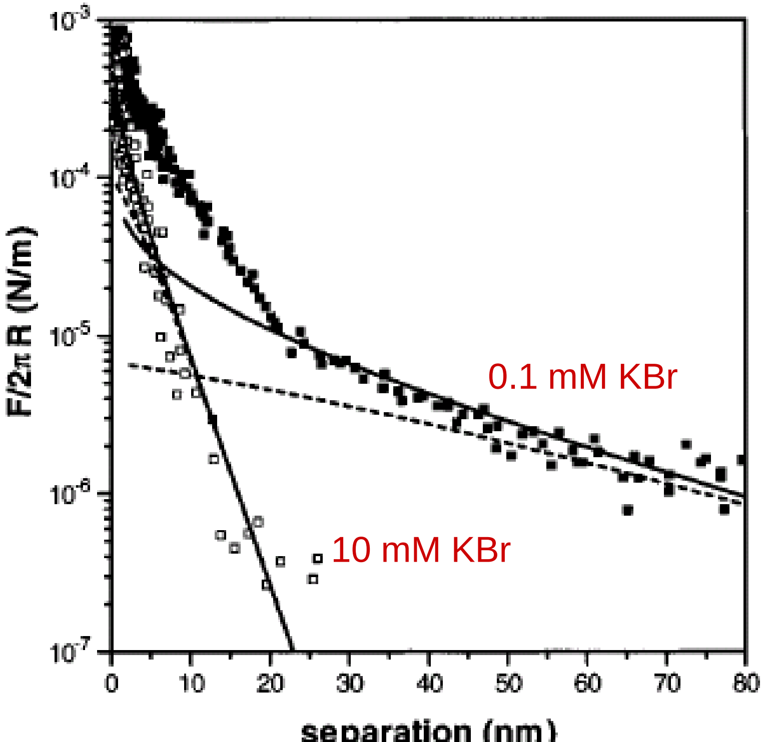
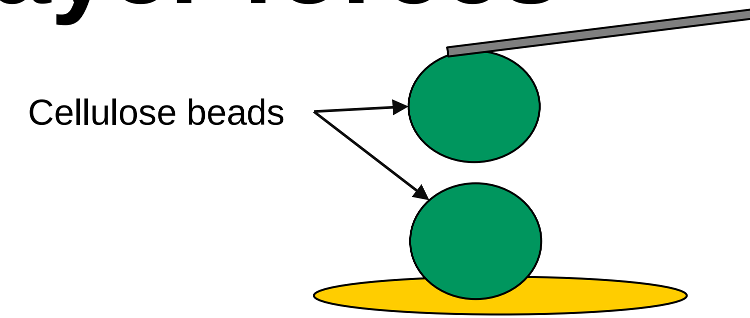
Electrostatic double layer forces



Carambassis and Rutland Langmuir, 1999



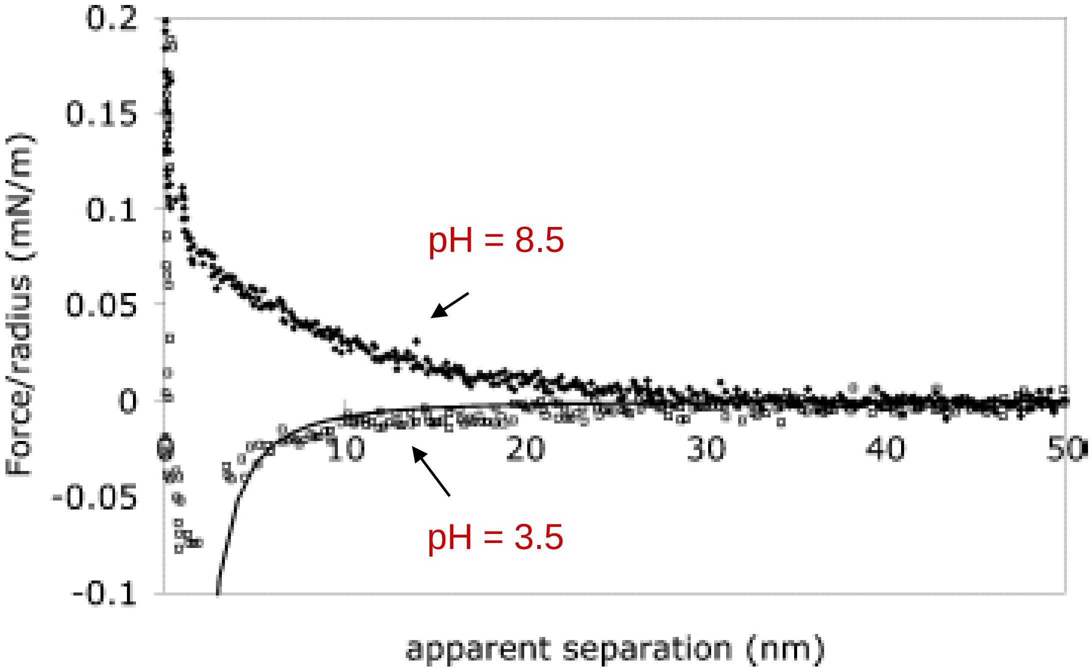
Electrostatic and steric forces were observed

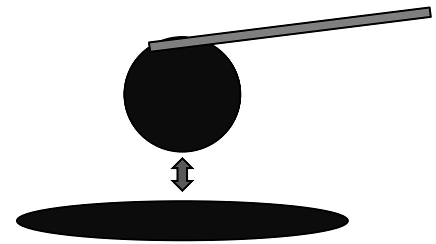
pH about 7

Why do we not observe van der Waals attraction at short distances?

Steric repulsion at short separation

Van der Waals forces between cellulose surfaces



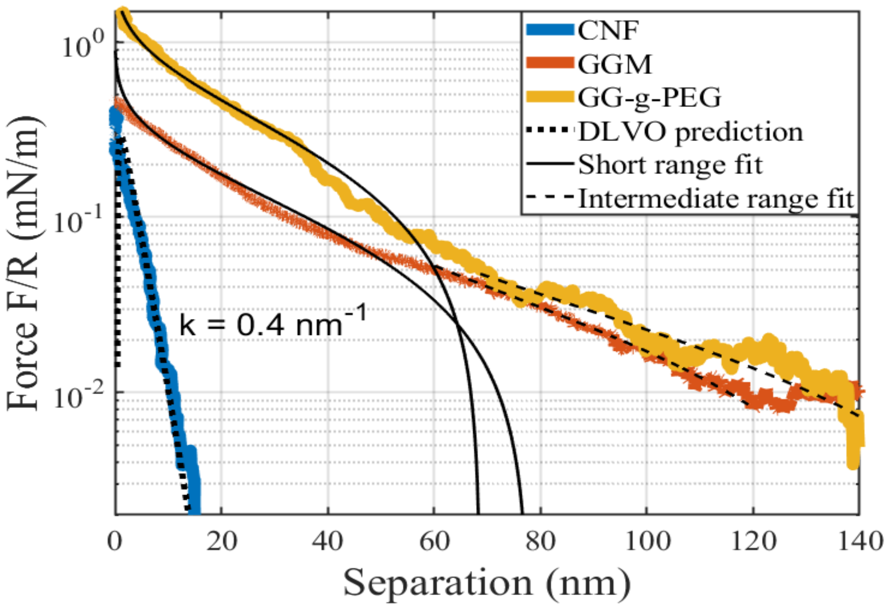


One cellulose sphere against a cellulose film, 1 mM NaCl

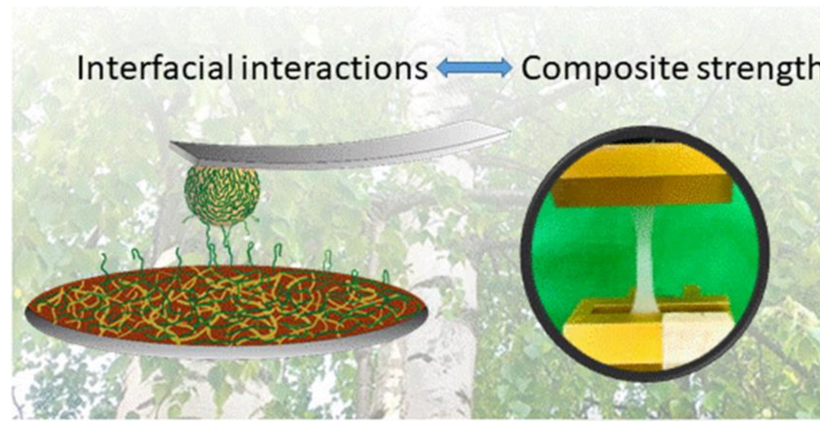
Why purely attractive at low pH and repulsive forces at higher pH?

Notley et al., Langmuir 2006 https://pubs.acs.org/doi/10.1021/la052886w

Polysaccharides adsorbed onto cellulose – Steric forces



Brush length of adsorbed polymer layer and correlation to mechanical properties



CNF –cellulose bead before adsorption:
DLVO prediction ok
For the other systems: mainly steric forces due to adsorbed polysaccharides

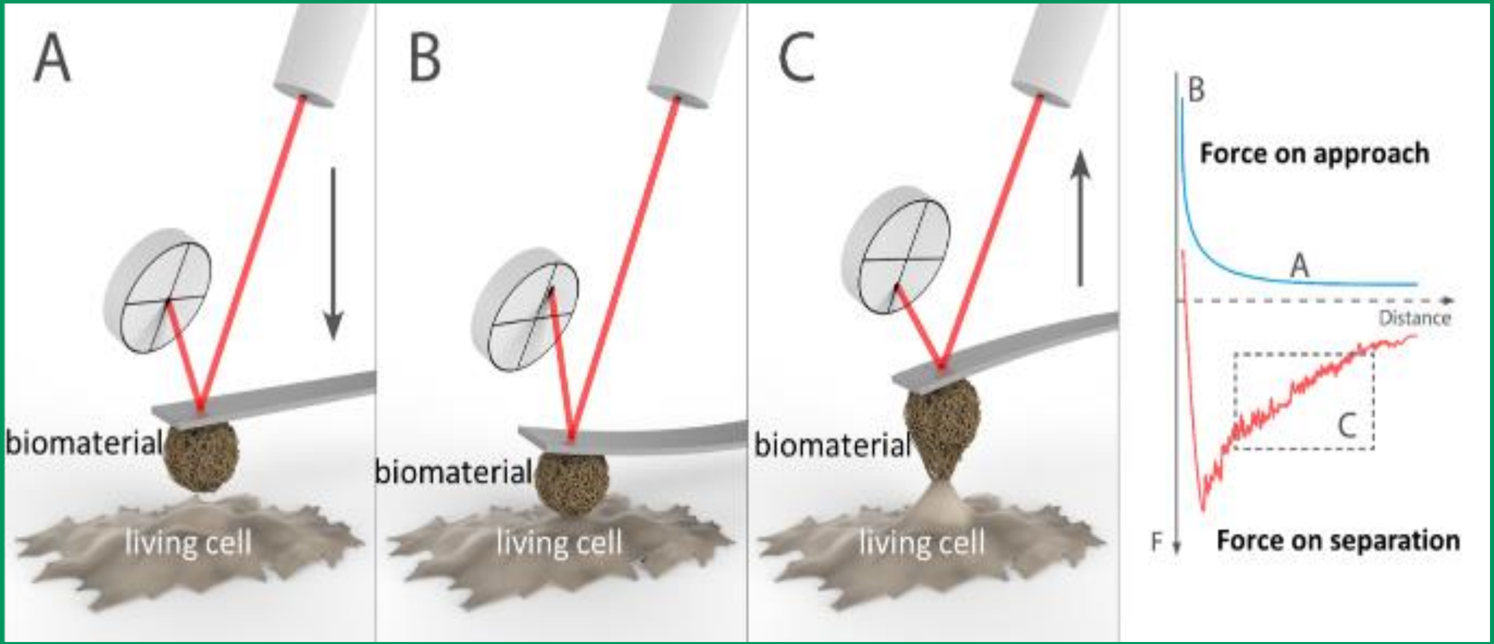
GGM = galctoglucomannan
GG-g-PEG = guargum grafted with polyethylene glycol

Lucenius et al JCIS 2019

Some reflections

- Force curve and effect of ionic strength tells us what forces dominate
- We can determine surface potential via fitting to DLVO theory
- We can estimate structure of adsorbed polymer layer, like brush length or brush vs mushroom structure via fitting to polymer brush model

Interactions between living cells and biomaterials



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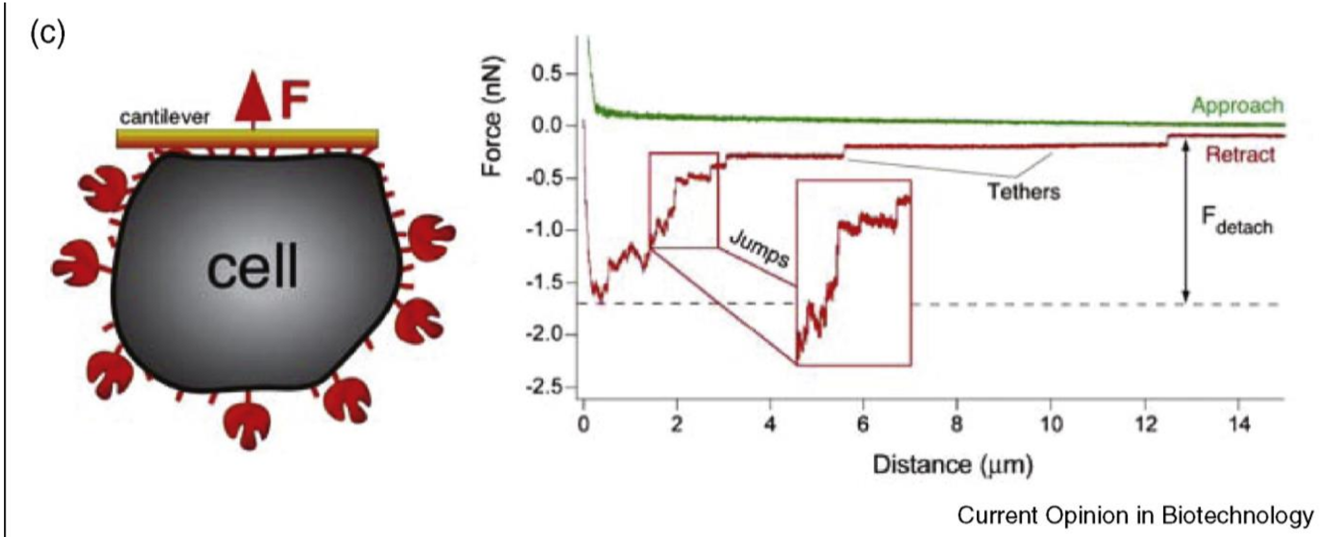
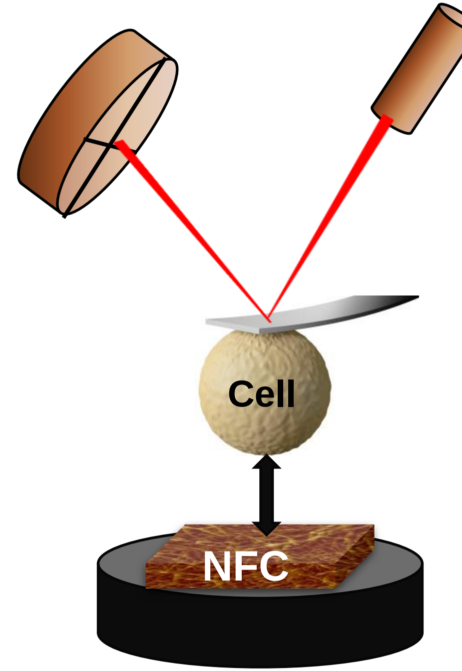
Commonly used method: Single cell force spectroscopy





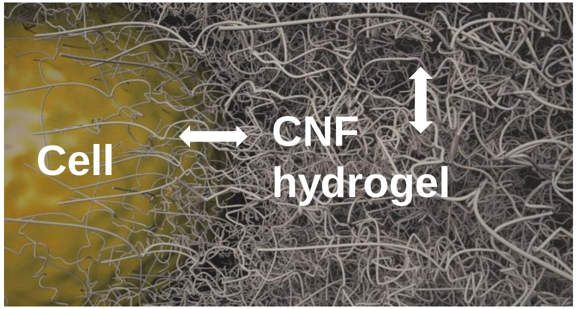
Muller et al https://doi.org/10.1016/j.copbio.2009.02.005

SCFS gives understanding of:
Molecular mechanism of cell adhesion
Specific and non specific interactions

Forces between stem cells and biomaterials

Challenge: stem cells cannot survive alone -> Colloidal probe approach

- **To give tools for better material design for tissue engineering**
- **To correlate direct interactions forces between living cells and biomaterials with phenomena found during cell culture *in vitro***



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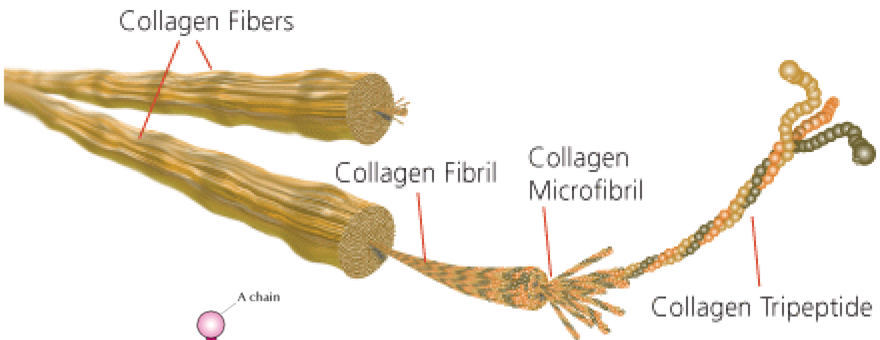
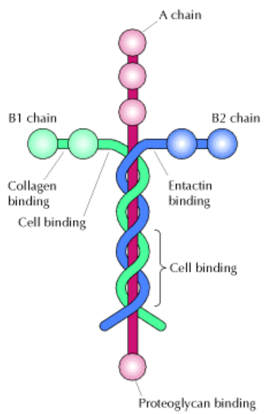
Choice of biomaterials

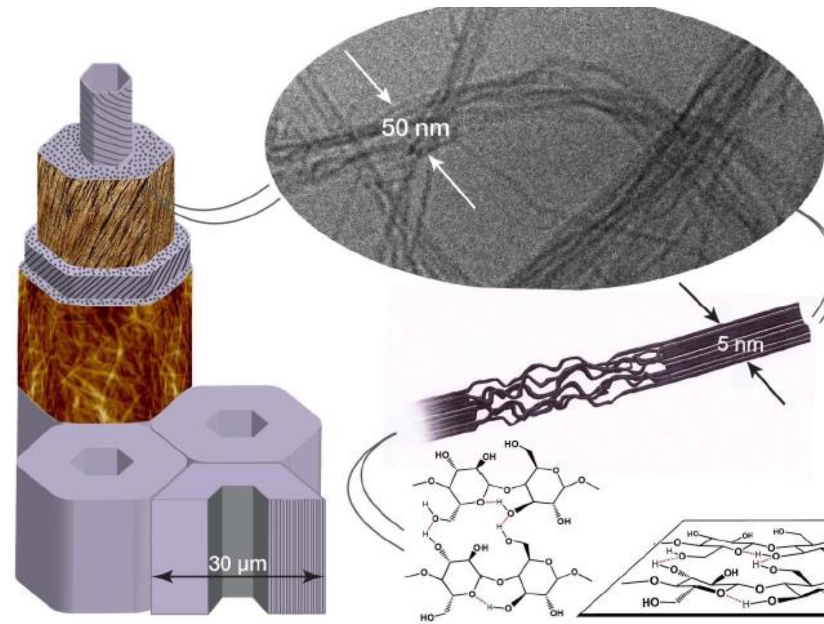
Collagen I and IV





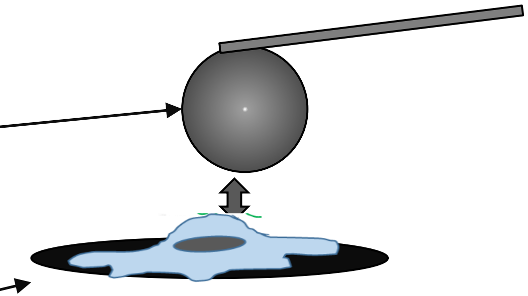
Laminin

Cellulose nanofibrils from wood (CNF)

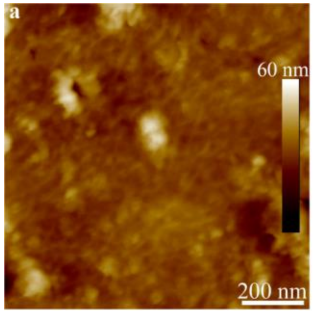


Fiber: width~ 30 µm, length 1-3 mm

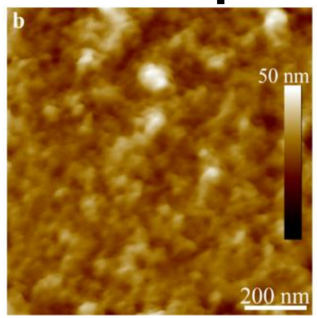
The system



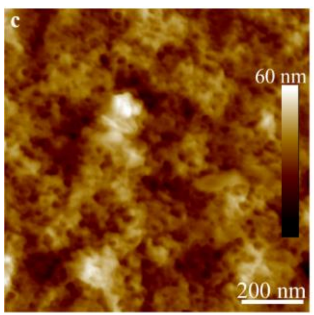
Biomaterials coated on probe



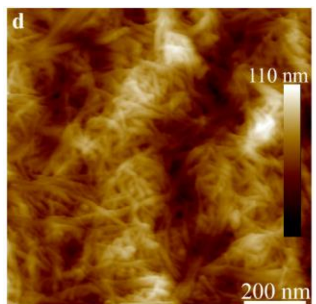
Collagen I



Collagen IV



Laminin 521

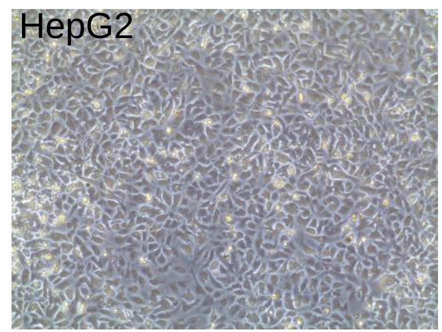


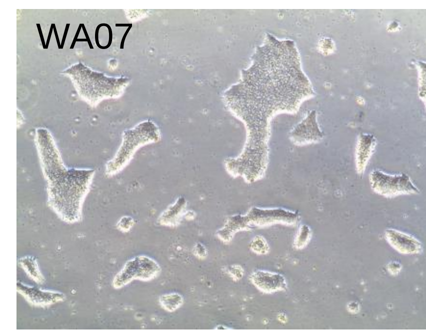
CNF (Growdex®)

Cell types

WA07 (human embryonic stem cell line)

HepG2 (human hepatocellular carcinoma cells)



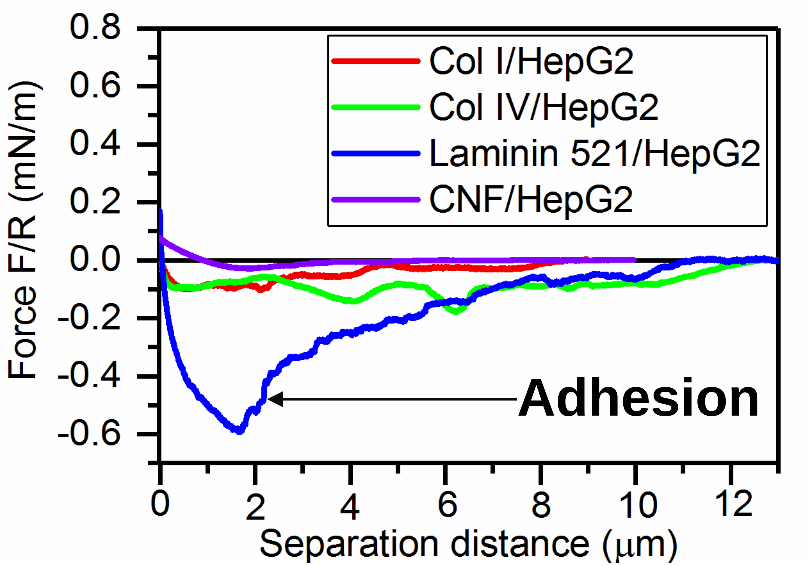


Conditions: Phosphate buffer saline with added Ca^{2+}, Mg^{2+} (PBS+), 37 ^oC

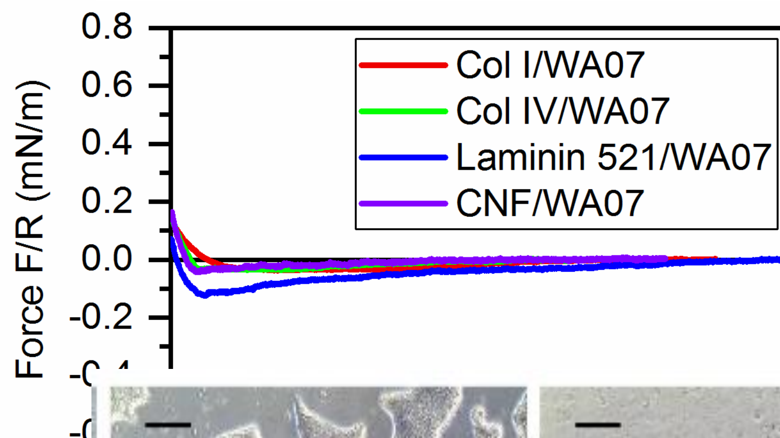
Interactions between *living* cells and biomaterials – Effect of cell line

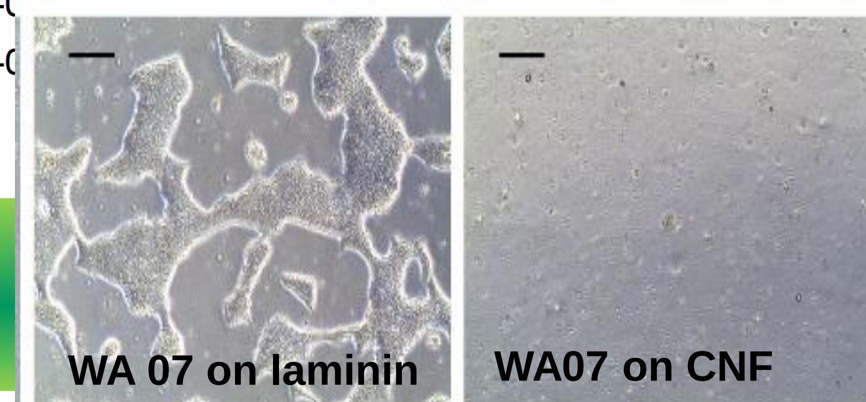
30s in contact
Retraction curves

HepG2 (carcinoma cells)



WA07 (stem cells)





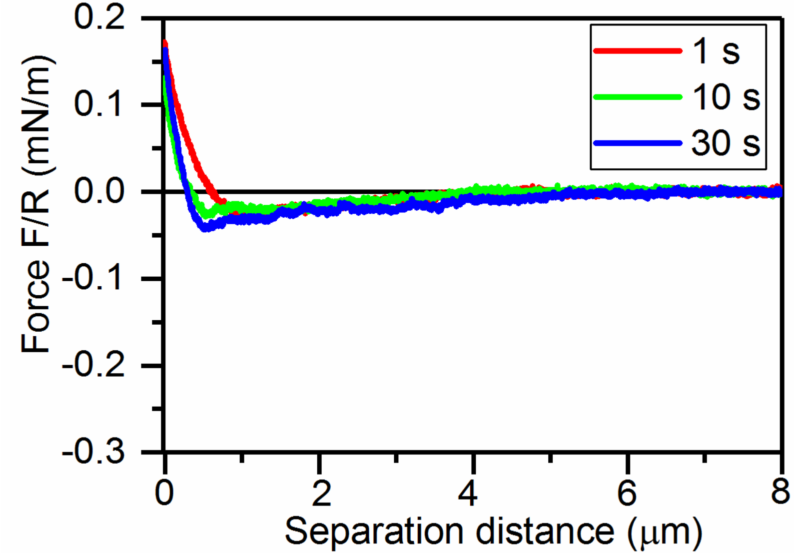
Very long ranged adhesion
Stem cells have weaker interaction than commonly used cancer cells

Harjumäki et al Scientific Reports 2019

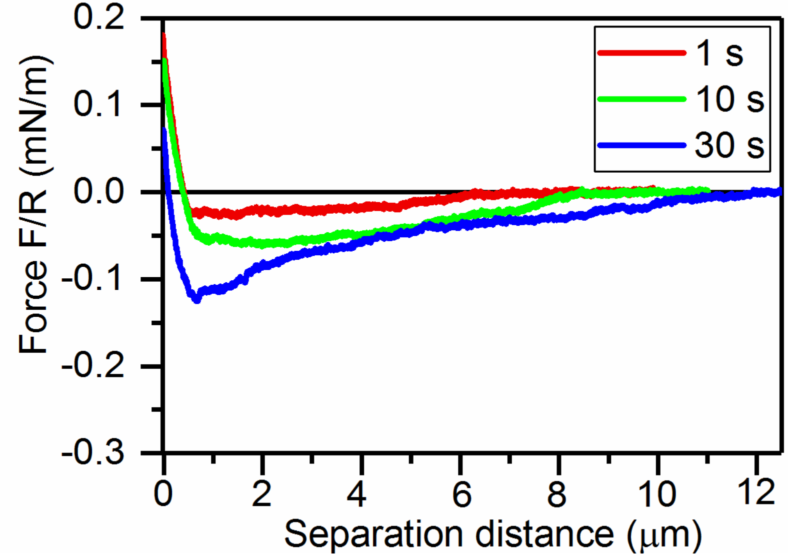
Effect of time in contact

Retraction curves

Living WA07/CNF



Living WA07/Laminin 521



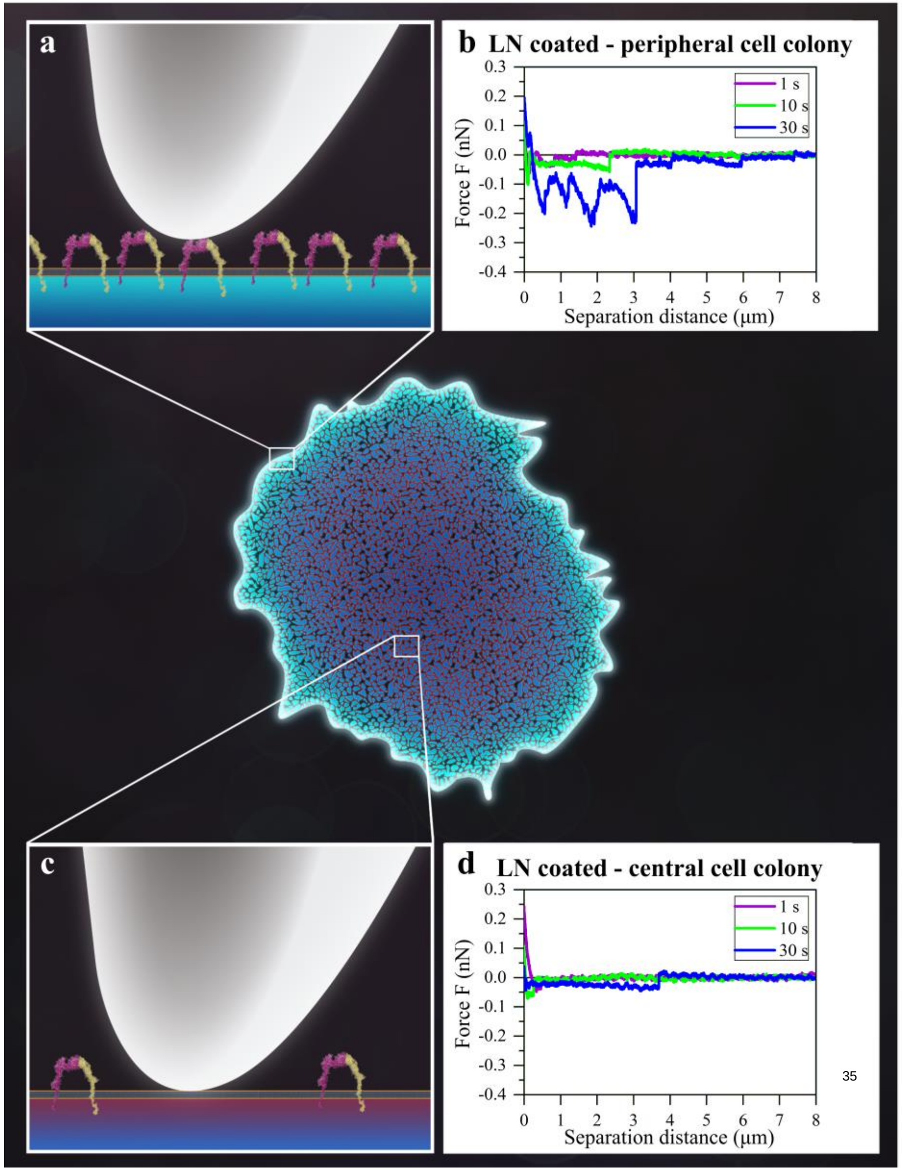
WA07 (stem cells)

Harjumäki et al Scientific Reports 2019

Specific vs nonspecific forces

- Living cells on the substrate (human pluripotent stem cells)
- Well defied tip coated with laminin
- Strong interaction between activated integrins at the cell membrane and the laminin
- Different force curve profile -> the integrins are more concentrated at the periphery of cell colonies

Harjumäki et al ACS Applied Biomaterials 2020

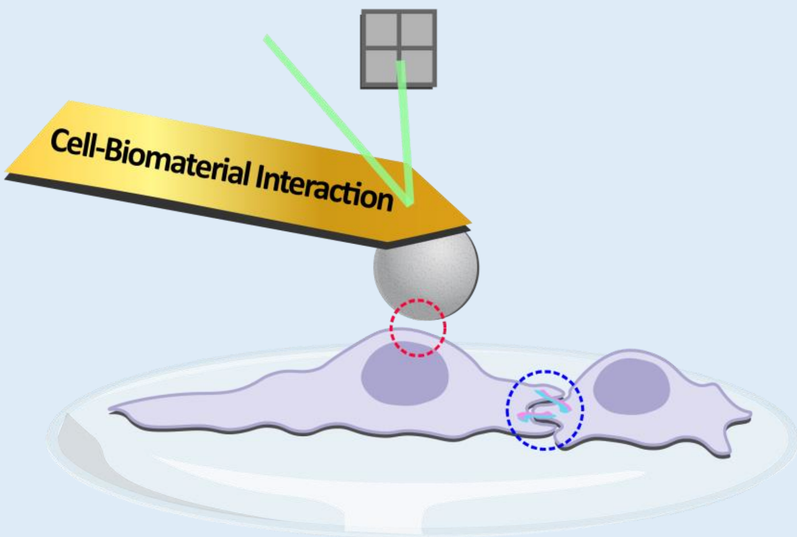


Take home message from previous example

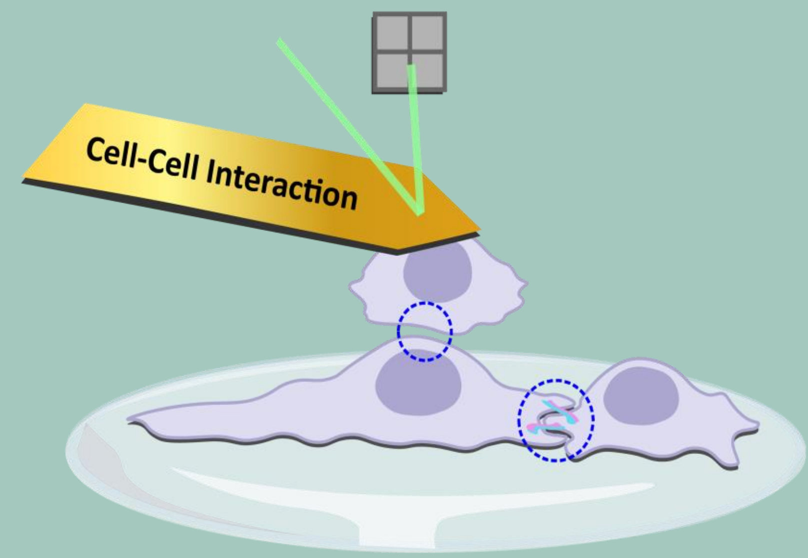
- Colloidal probe method suitable for delicate cells
- Specific and unspecific interactions can be distinguished
- Good correlation between phenomena found during in vitro cell culturing and direct force measurements
- Cell – biomaterial interactions can be controlled with proteins

Correlation between cell-material interactions and spheroid formation (using the Bio-AFM at the department)

Colloidal Probe Microscopy (CPM)



Single Cell Force Spectroscopy (SCFS)



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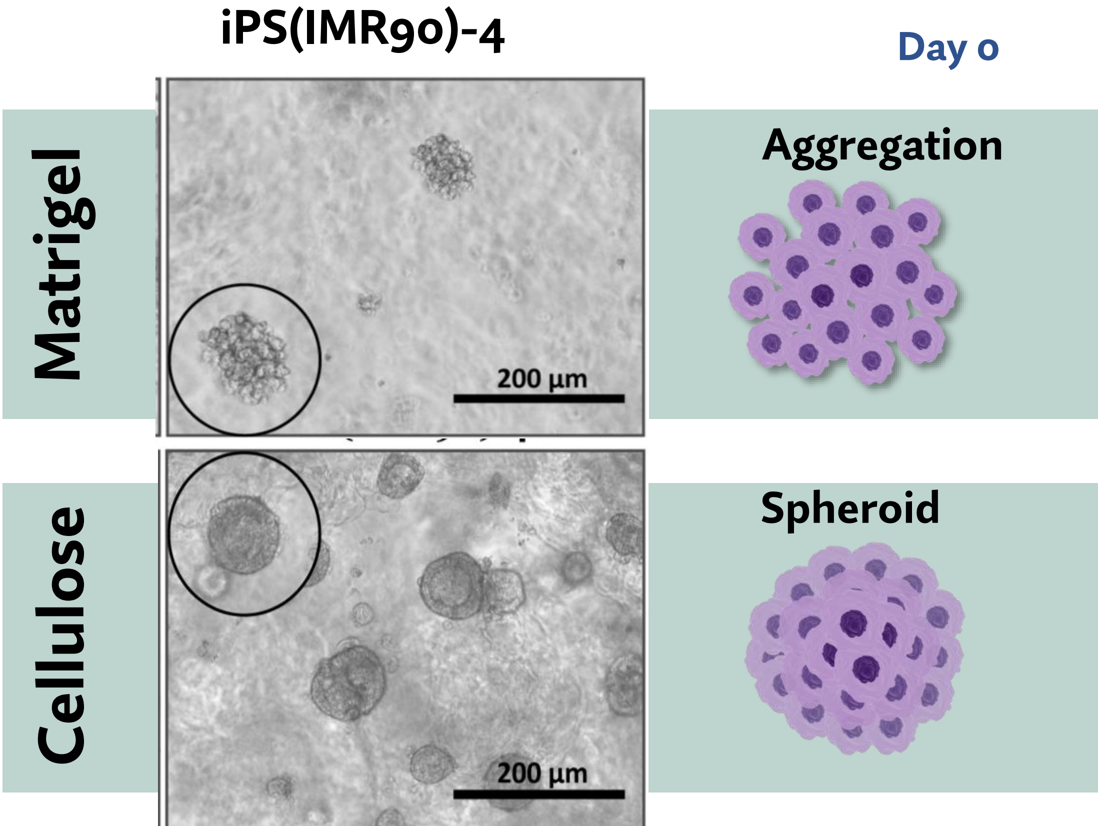

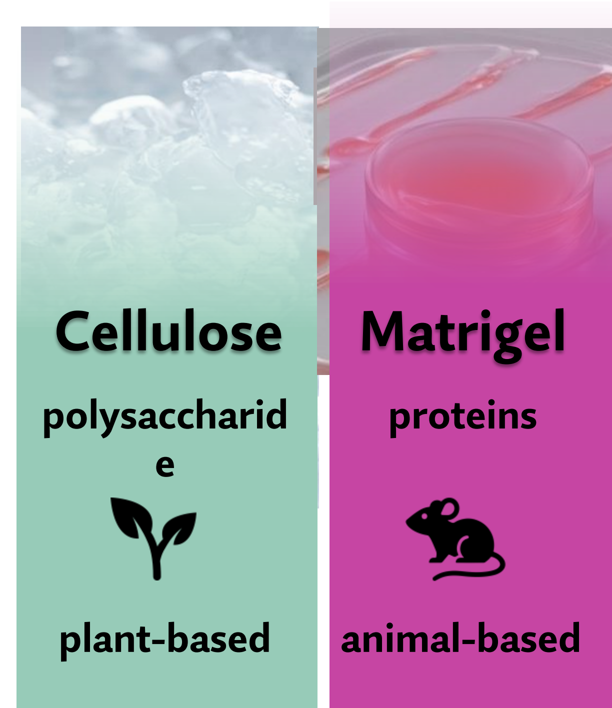

CELL LINES:
Liver cancer (HepG2)
Stem cell (iPS(IMR90)-4)

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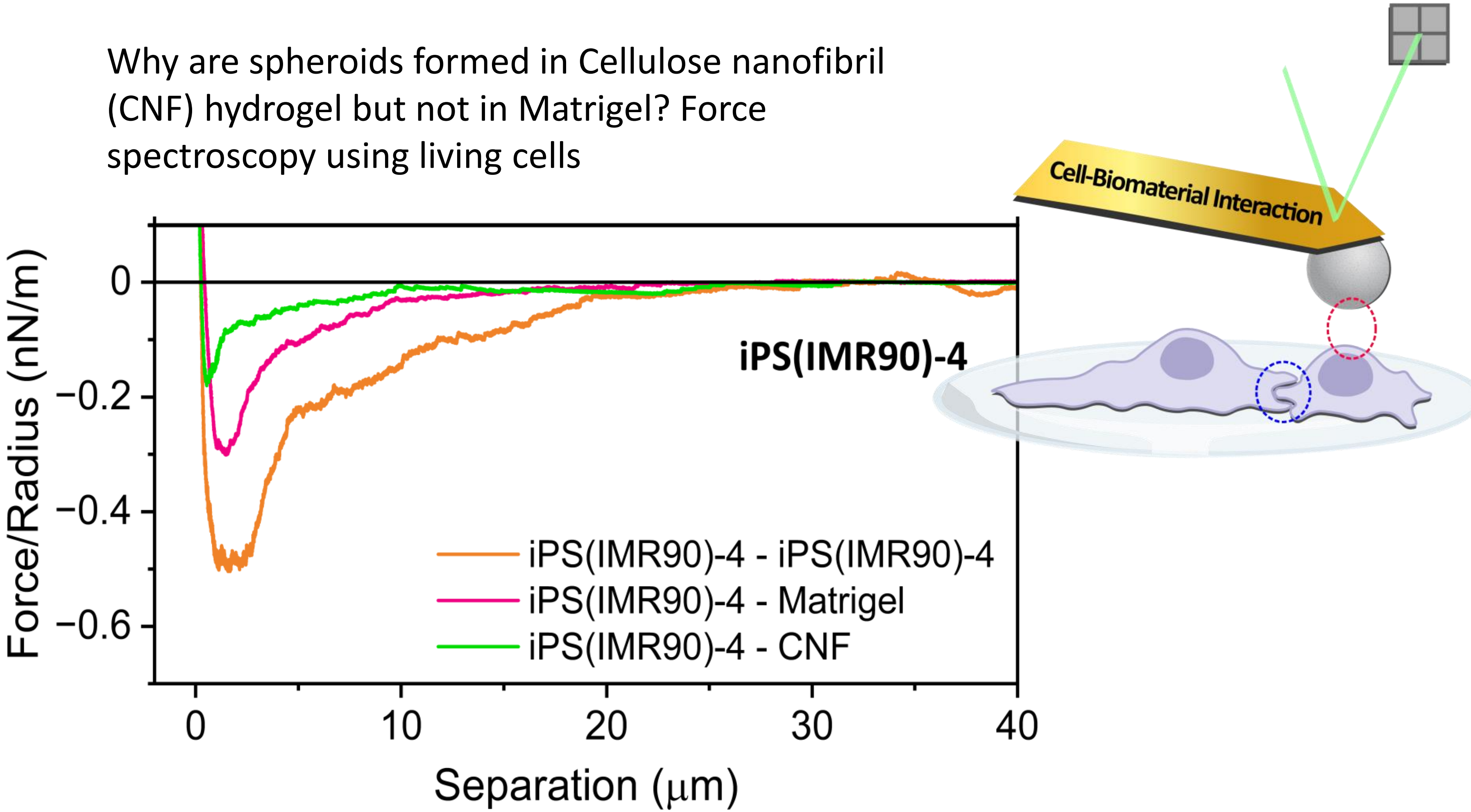
Why are spheroids formed in Cellulose nanofibril (CNF) hydrogel but not in Matrigel? Force spectroscopy using living cells



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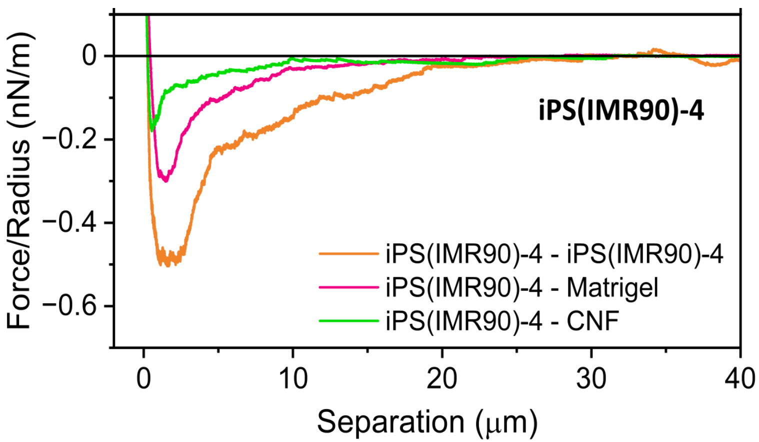
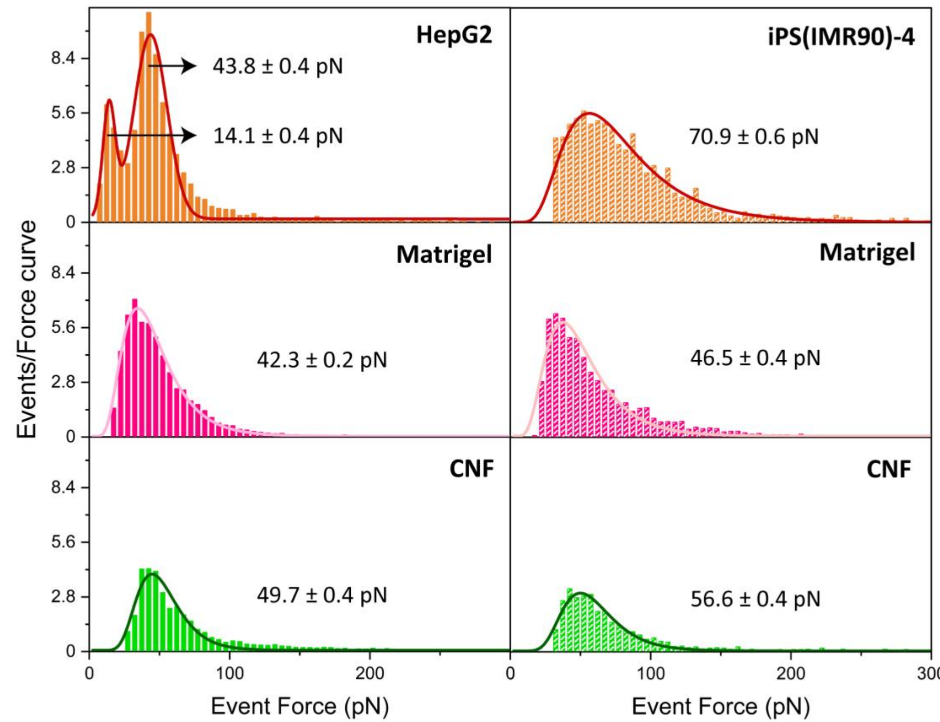
Analysis of distribution of measured bond rupture events





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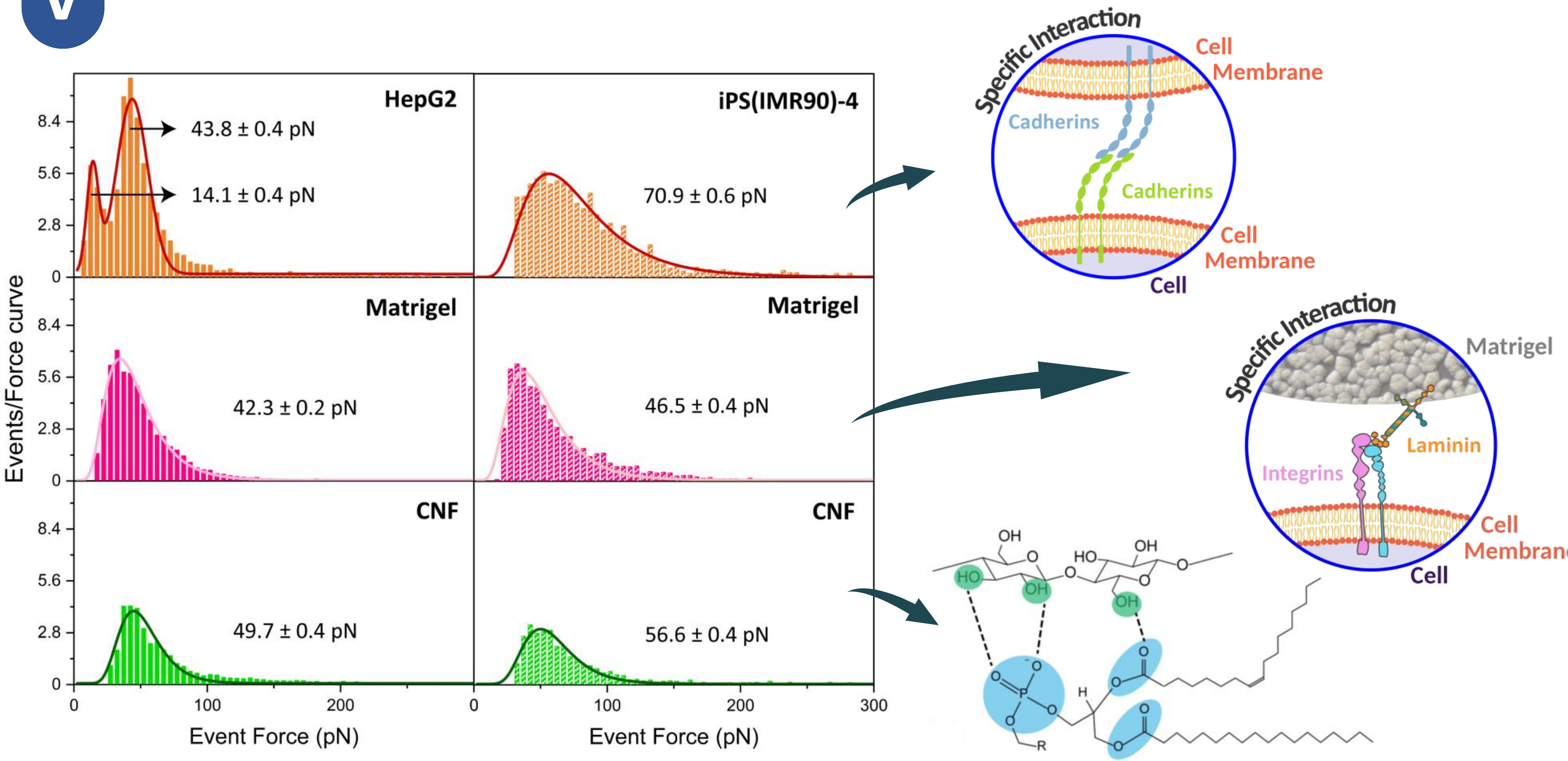
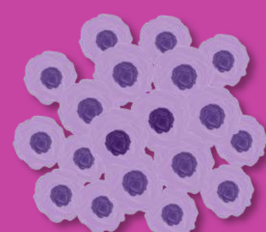

HepG2
43.8 ± 0.4 pN
14.1 ± 0.4 pN
iPS(IMR90)-4
70.9 ± 0.6 pN
Matrigel
42.3 ± 0.2 pN
Matrigel
46.5 ± 0.4 pN
CNF
49.7 ± 0.4 pN
CNF
56.6 ± 0.4 pN
Events/Force curve
Event Force (pN)
Event Force (pN)
Specific Interaction
Cell Membrane
Cadherins
Cadherins
Cell Membrane
Cell
Specific Interaction
Matrigel
Laminin
Integrins
Cell Membrane
Cell

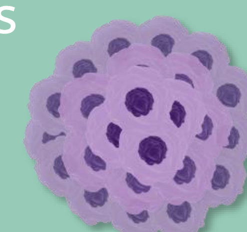
Cells in Matrigel

- Downregulate cell-cell interaction
- Relied on integrin-laminin interaction
- Cells stay scattered



Cells in Cellulose

- Upregulate cell-cell interaction
- No specific interaction
- Spheroids formed



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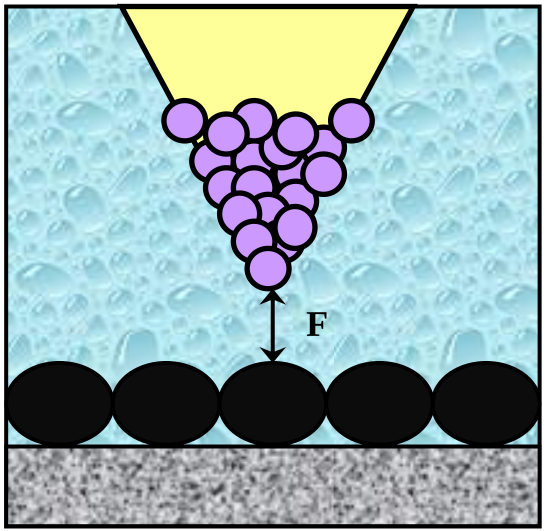
What are the main challenges when measuring forces using living cells?

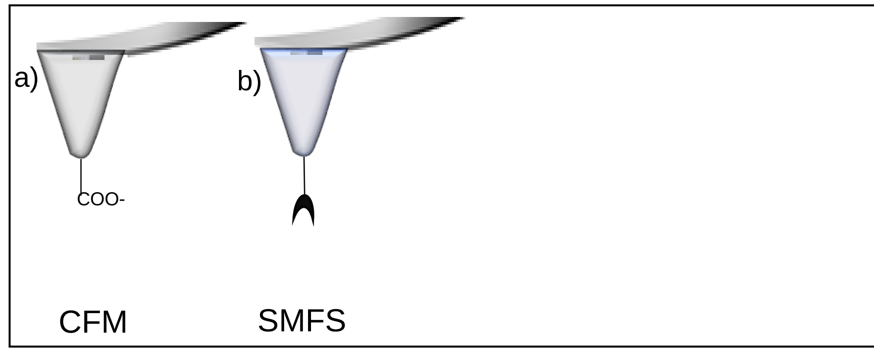
- **Large variation in results**
- **Cells delicate and soft**
    - Affected by force measurements
    - Radius, contact area or constant compliance region difficult to determine

How can these challenges be overcome/mitigated?

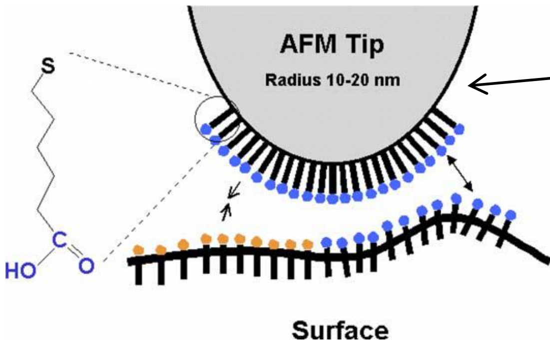
Try to use cells of similar age, make many parallel measurements
Be careful with the handling of cells, control conditions during measurements, let cells relax in between measurements, monitor viability during and after measurements, measure only as long as cells are still doing well

Chemical Force Microscopy and Single Molecule Force Spectroscopy

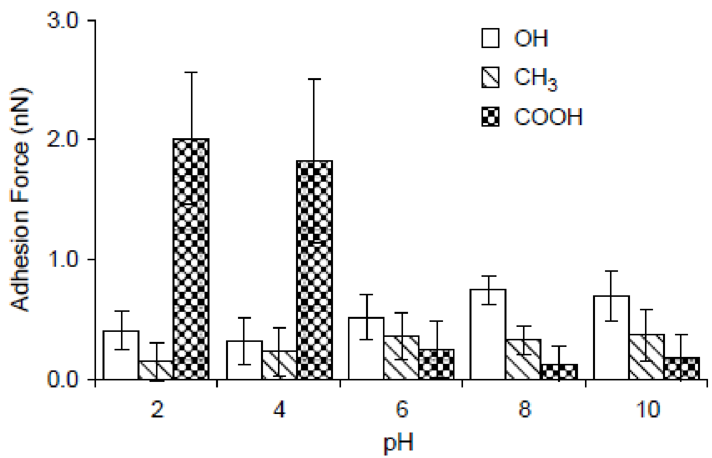




Chemical force spectroscopy: Example interactions with cellulose pulp fibre surface



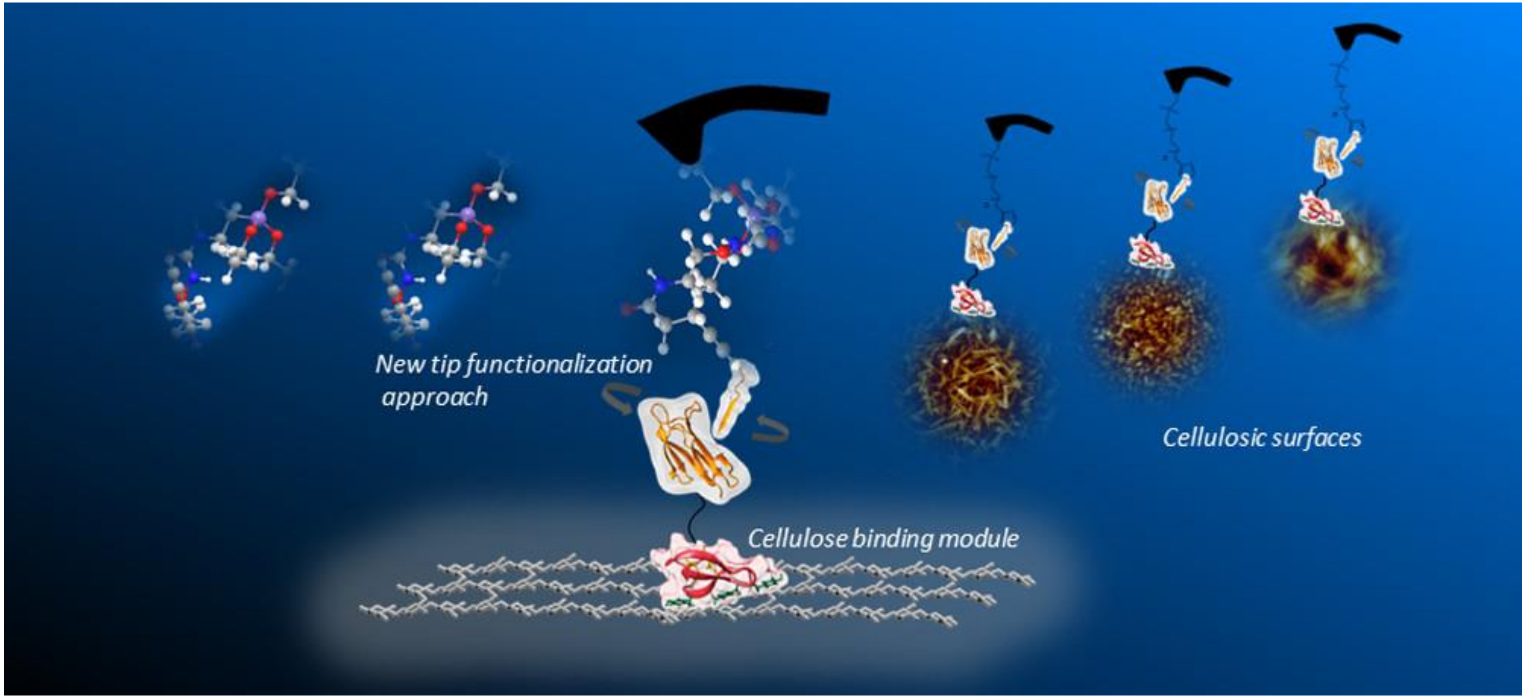
SAMS with –OH , – CH_3 or –COOH



- Highest adhesion between COOH and cellulose
- pH dependence
- Almost no adhesion between CH_3 and cellulose

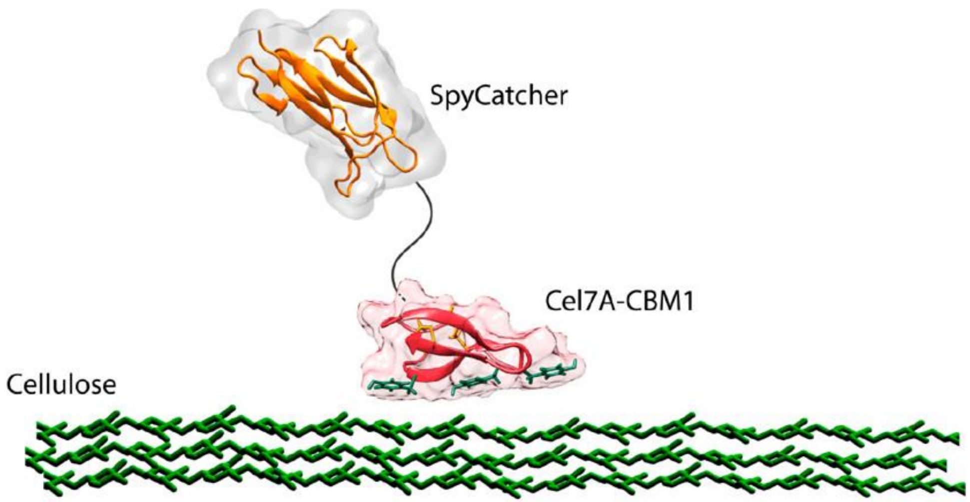
Bastidas et al Carbohydrate Polymers 62(2005) 369

Binding forces of cellulose binding modules (CBD) on cellulosic nanomaterials



Griffo et al Biomacromolecules 2019

Tip is functionalized with cellulose binding modules (CBD)

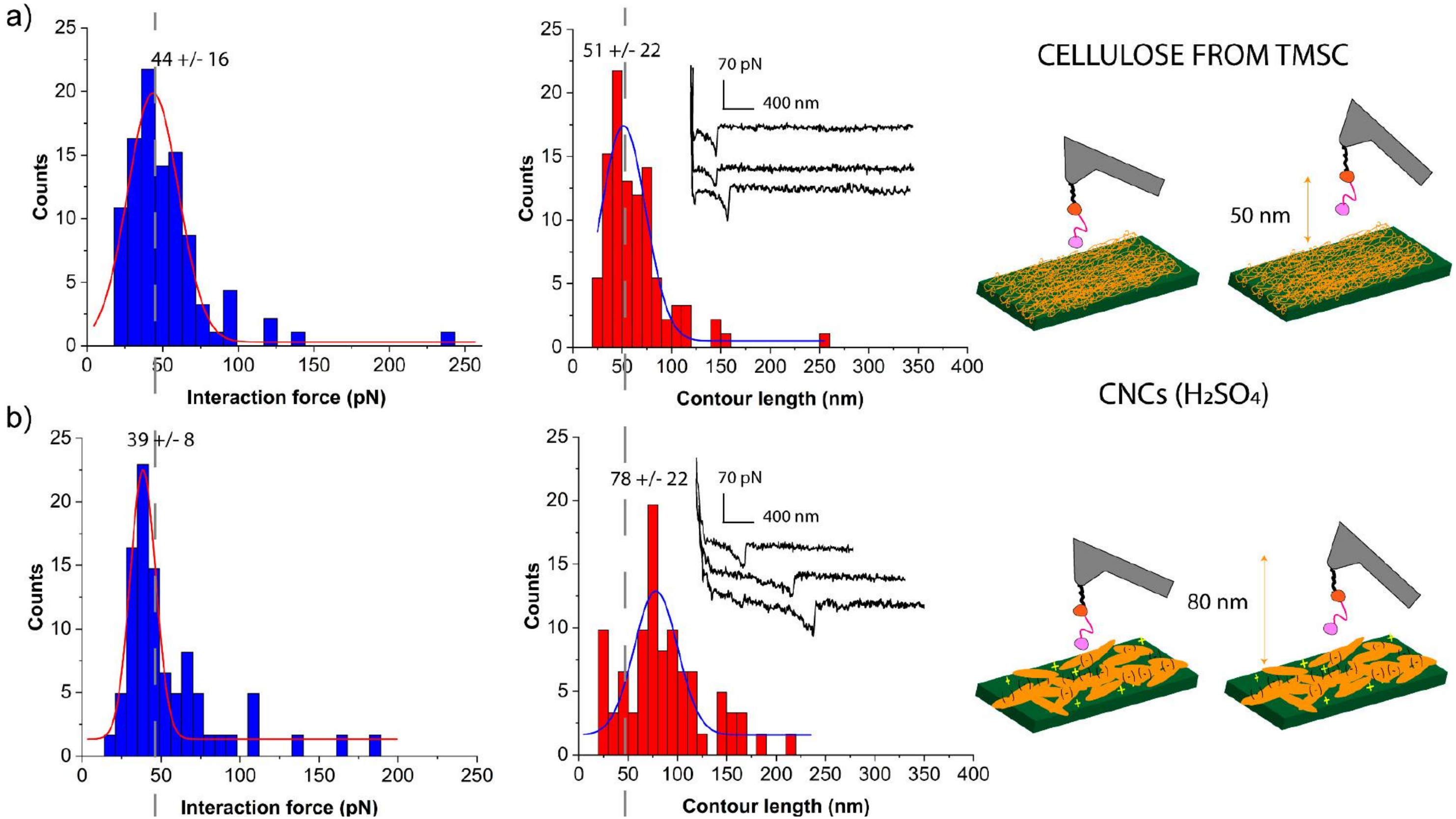


Interaction forces against different types of cellulose substrates: Cellulose from TMSC, CNCs from sulphric acid hydrolysis, CNCs from HCl hydrolysis, and Chitin nanocrystals, C

- SpyCatcher-Cel7A-CBM1 had a similar interaction force on the surfaces of unmodified cellulose, although they varied by the degree of crystallinity and morphology.
- Focus on the tip modification method

Griffo et al Biomacromolecules 2019

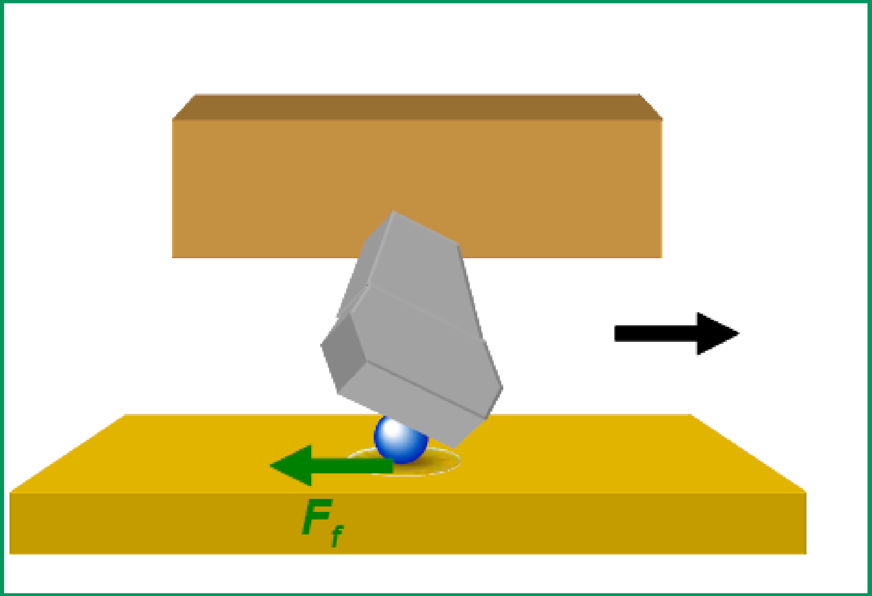
Effect of cellulosic material on the binding force between CBM and cellulose



Contour length – via fitting to wormlike chain model for semiflexible polymer chain

Griffo et al Biomacromolecules 2019

Friction force measurements



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Friction – what is it?

One should distinguish between two different regimes:

Hydrodynamic (liquid) friction

- the substrates are separated by a thick (> 0.01 mm) liquid film
- friction mainly determined by viscosity of liquid lubricant

Boundary lubrication

- the substrates are separated by a thin (a few atomic diameters) lubricating film
- also dry friction

Friction is the resistance to motion during sliding or rolling of a solid body against another.

The force acting in the direction opposite to the direction of motion is called friction force

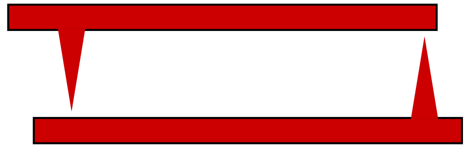
Friction

Amontons law: *F* (friction force) = μL

μ= friction coefficient, L = load

$F_1 = F_2$ i.e. no dependence on contact area!

What about surface roughness??

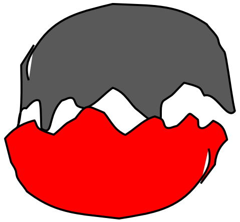


Since friction usually is affected by roughness we need to seek an explanation which involves adhesion.
This requires that surface area is important **BUT** Amontons law tells us that friction depends only on load

Is there a load – area relationship?

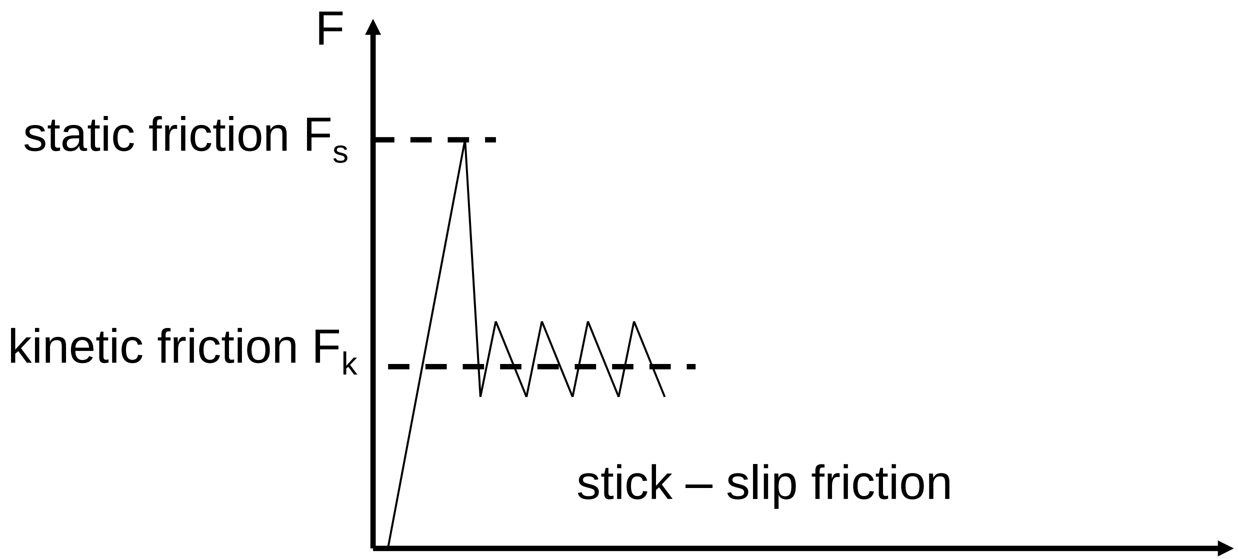
The real contact area is usually much smaller than the geometrical area

For soft samples the real area is dependent on load => Amontons law



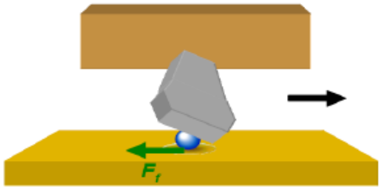
A fundamental understanding of adhesion and friction requires an understanding of the mechanisms on the atomic/molecular scale =>Friction force measurements with AFM or SFA

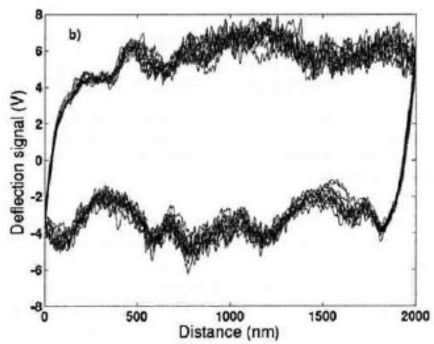
Kinetic versus static friction

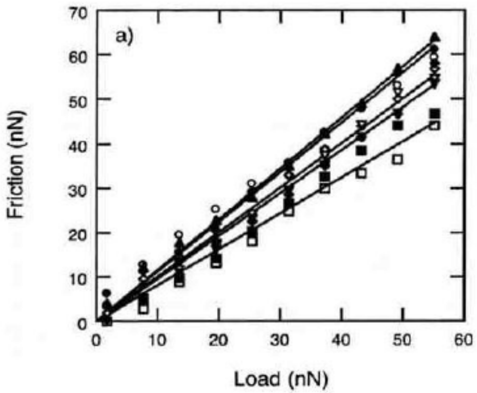


The static friction force is always larger than the kinetic friction force

Friction force measurements using AFM

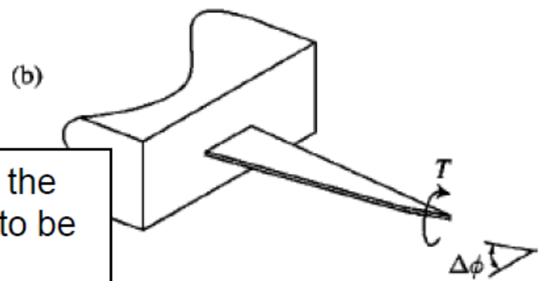






- ➢Friction loops at different loads are measured
- ➢Friction as a function of load
- ➢ The slope → Friction coefficient (μ)

Amontons law: *F* (friction force) = *μL*



For friction force measurements the torsional spring constant needs to be determined

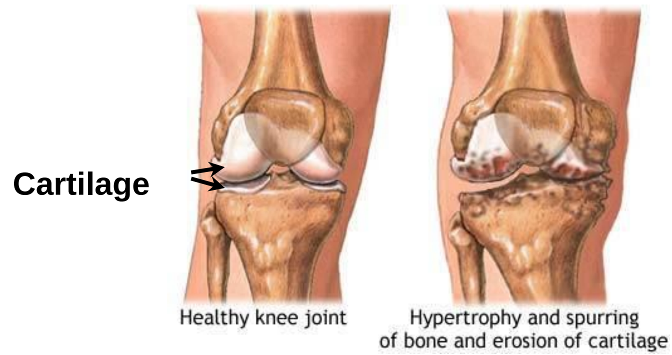
Example Bioinspired lubricating films of cellulose nanofibrils and hyaluronic acid

Background

Articular cartilage, a flexible tissue that protect the bones in the joints, has a **limited capacity for self-repair**.

Main requirements:

- Able to withstand high applied loads
- High resistance to wear
- Low friction coefficient (lubrication)
- Biocompatible



Cartilage damage and cartilage-related diseases:

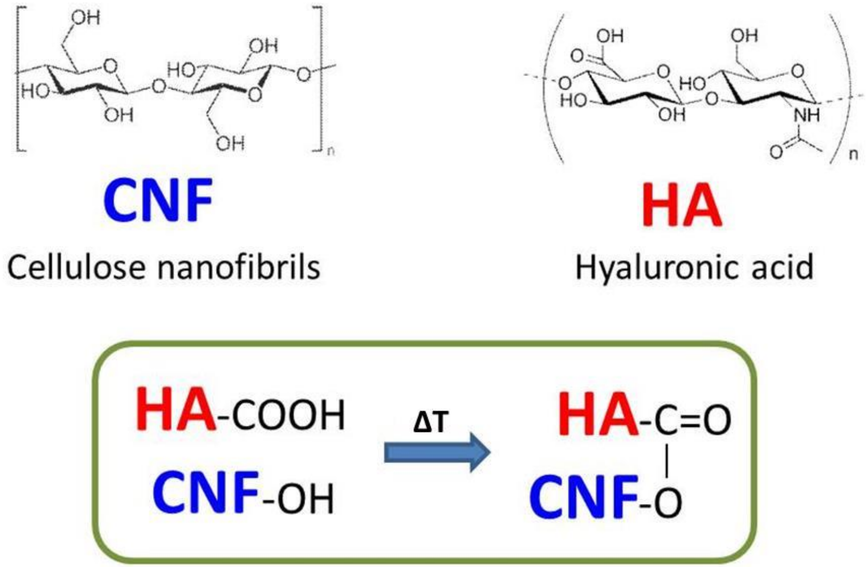
Europe: about **25 million people** suffer from osteoarthritis; **90 million EUR/year**.

Approach

- **Hyaluronic acid (HA) is a natural lubricating polymer present in the joints.**
- **Hyaluronic acid (HA) has low mechanical properties**
- **CNF films have very good mechanical properties but high friction**

Combining the mechanical strength of CNF with the lubricating effect of HA

Valle-Delgado, JJ, Johansson, L-S, Österberg, M, (2016) *Colloids and Surfaces B: Biointerfaces* 138 86-93.



HA attached to CNF films by esterification reaction between hydroxyl and carboxyl groups

Objective:
Durable lubricating layer

Valle-Delgado, JJ, Johansson, L-S, Österberg, M, (2016) *Colloids and Surfaces B: Biointerfaces* 138 86-93.

Normal forces – What forces are present?

Van der Waals Forces? Yes

Electrostatic Double-Layer Forces? Yes

Yes

Steric forces?

Increase in steric repulsion

What is the reason for the steric repulsion?

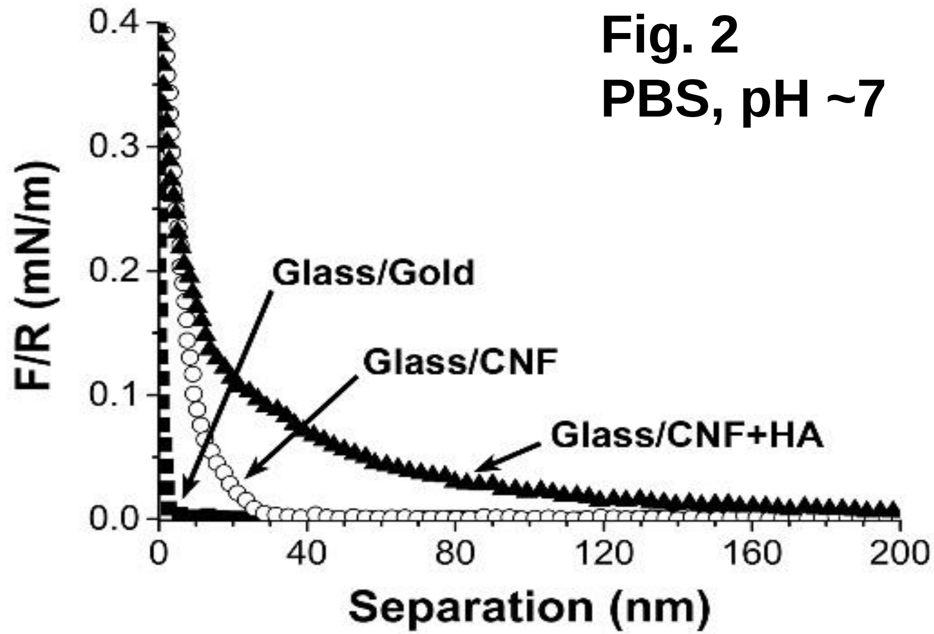


Fig. 2
PBS, pH ~7

PBS = phosphate buffered saline

dx.doi.org/10.1016/j.colsurfb.2015.11.047

Effect of ionic strength

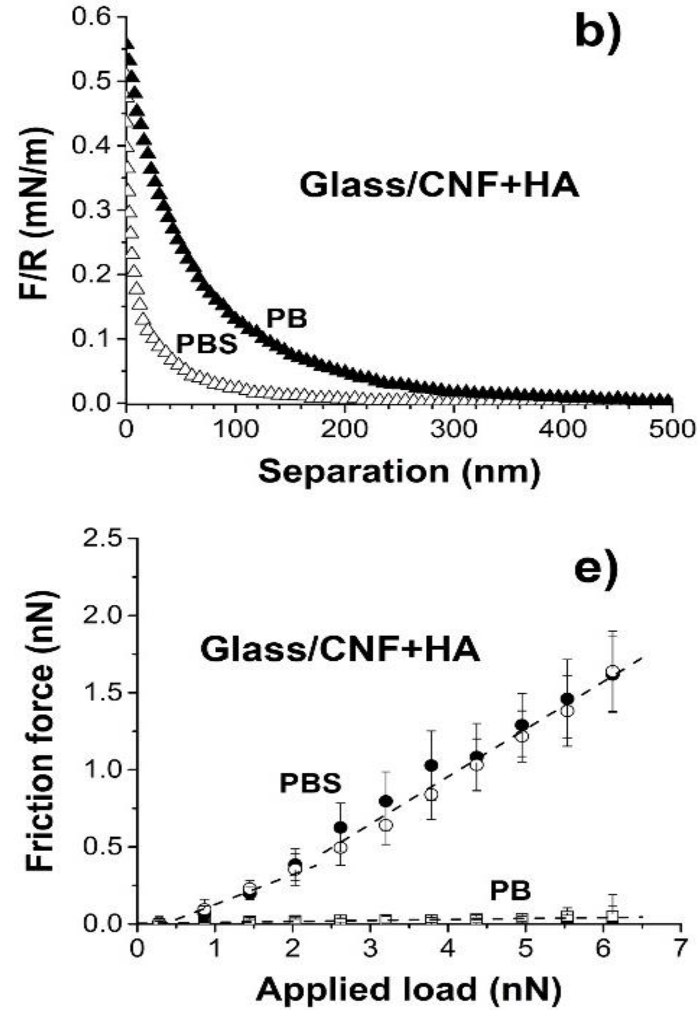
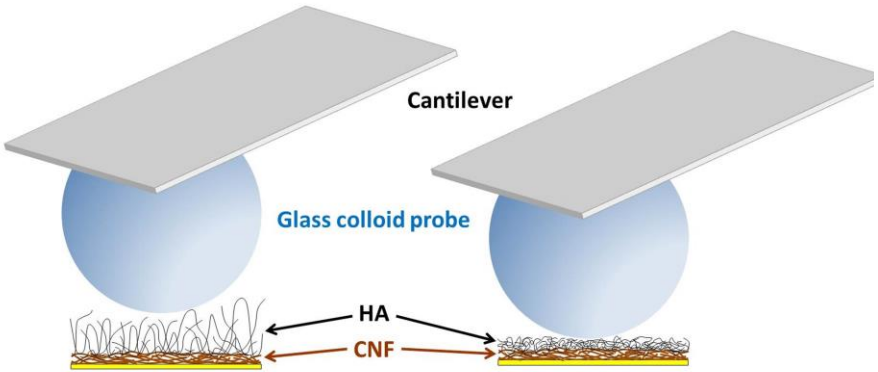


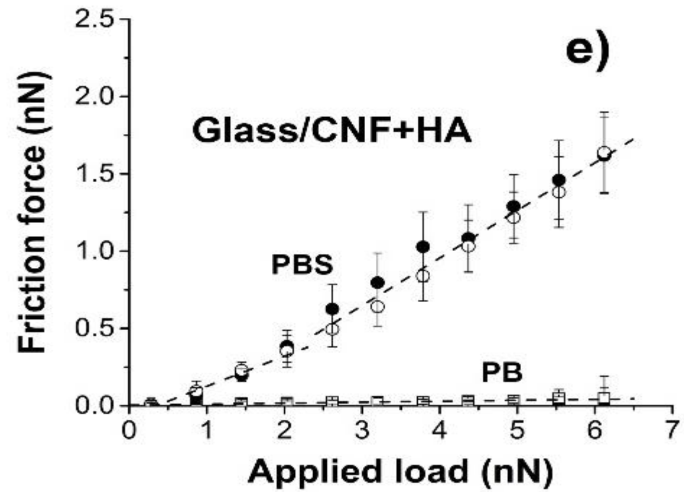
Fig 3. dx.doi.org/10.1016/j.colsurfb.2015.11.047

Hydrated layer, high repulsion
→ Low friction

Phosphate buffered saline (PBS):10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl **High I**
Phosphate buffer (PB): 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 **Low I**

Effect of ionic strength II





Low I
Hydrated fibril and polymer layers
Very low friction

High I
Collapsed polymer layer
Higher friction

dx.doi.org/10.1016/j.colsurfb.2015.11.047

Reflections on the previous example

Lubrication was achieved by attachment of polymers

Hydrated polymer layer

Extended polymer chains (good solvent)

Similar approach of surface modification can be used for:

- **Steric stabilization of nanoparticles**
- **Antifouling surfaces (grafting of PEG chains)**
- **In composites for better alignment of reinforcing fibers**

Take-home message

- **Interfacial properties are decisive in nanomaterials**
- **You gain understanding and can optimize your systems by doing direct surface force measurements**
- **Consider:** Differences and advantages between the different ways of modifying the tip
  - *Chemical force microscopy (CFM)*
  - *Single-molecule force spectroscopy (SMFS)*
  - *Colloidal probe microscopy (CPM)*
  - *Single cell force spectroscopy (SCFS)*