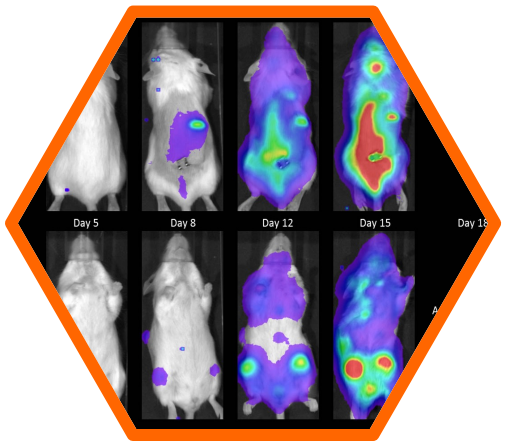
November 13th 2024

Biomaterials for MX

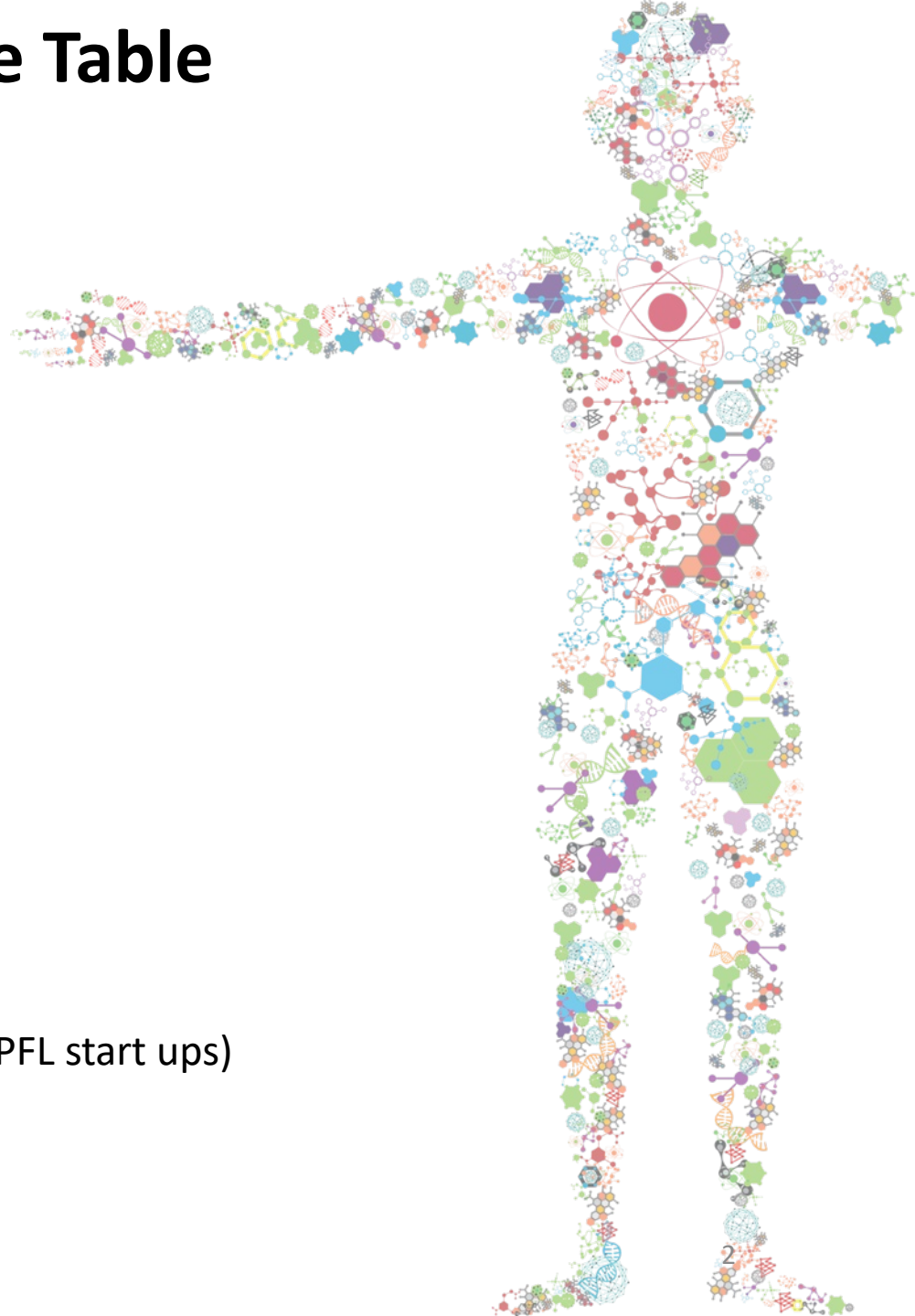
MSE – 471

Maartje Bastings



Course 9: Characterization and Performance

Course Content & Time Table



BLOCK 1: Introduction and materials overview

11-9	Lecture 1.	Intro to biomaterials and biology
18-9	Lecture 2.	Naturally derived biomaterials
25-9	Lecture 3.	Polymers and nanoparticles
2-10	Lecture 4.	Surfaces

BLOCK 2: Interactions and specific applications

9-10	Lecture 5.	Materials for drug delivery and targeting
16-10	Lecture 6.	Materials for cell adhesion
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30-10	Lecture 7.	Materials for immune engineering
6-11	Lecture 8.	Materials for tissue engineering

BLOCK 3: Measurements, regulation and translation

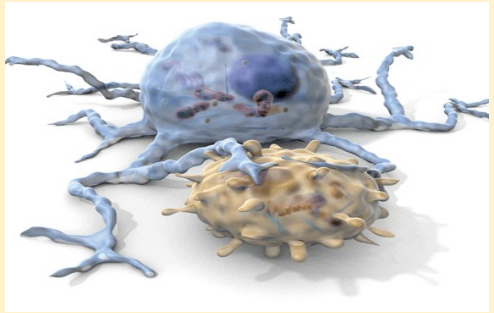
13-11	**Lecture 9.**	**Characterization and performance**
20-11	Lecture 10.	Sensors and diagnostic devices
27-11	Lecture 11.	Translation to industry, patents, spin-offs (EPFL start ups)
4-12	Lecture 12.	Regulatory aspects and trials (EPFL TTO)
11-12	Lecture 13.	Revision and conclusion
18-12	Open discussion and hand in of lab papers	

Characterization & Performance

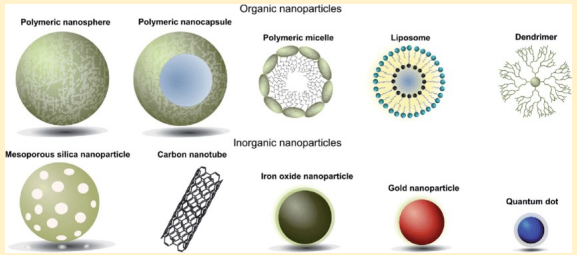
How to measure your materials properties?

Hydrogels

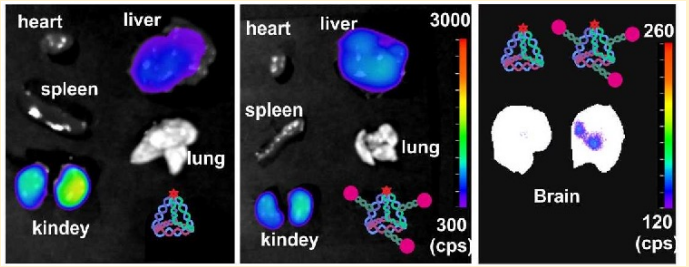
Immune response



Nanoparticles

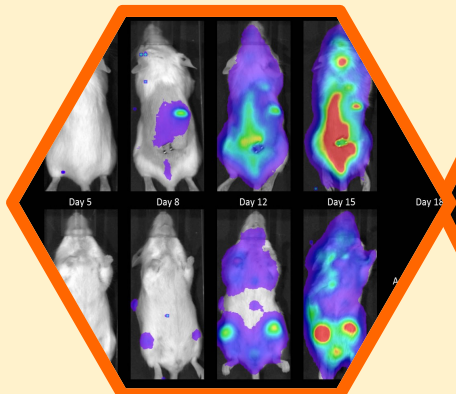


Targeting and drug release

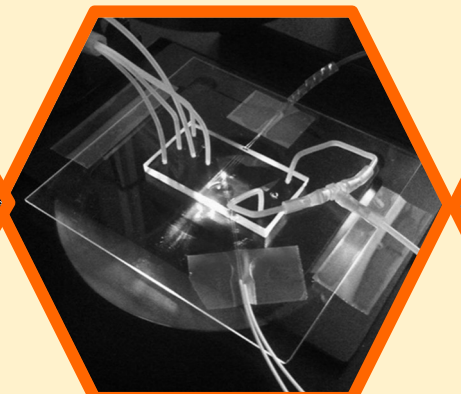


BLOCK 3: Quantification

Characterization



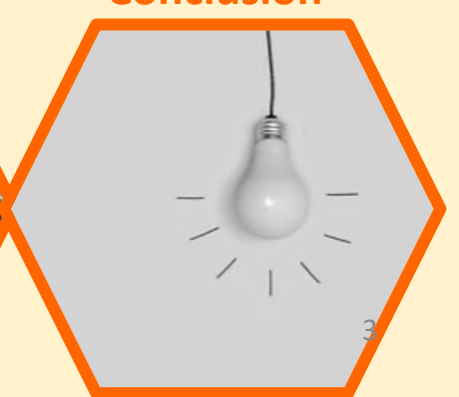
Sensors



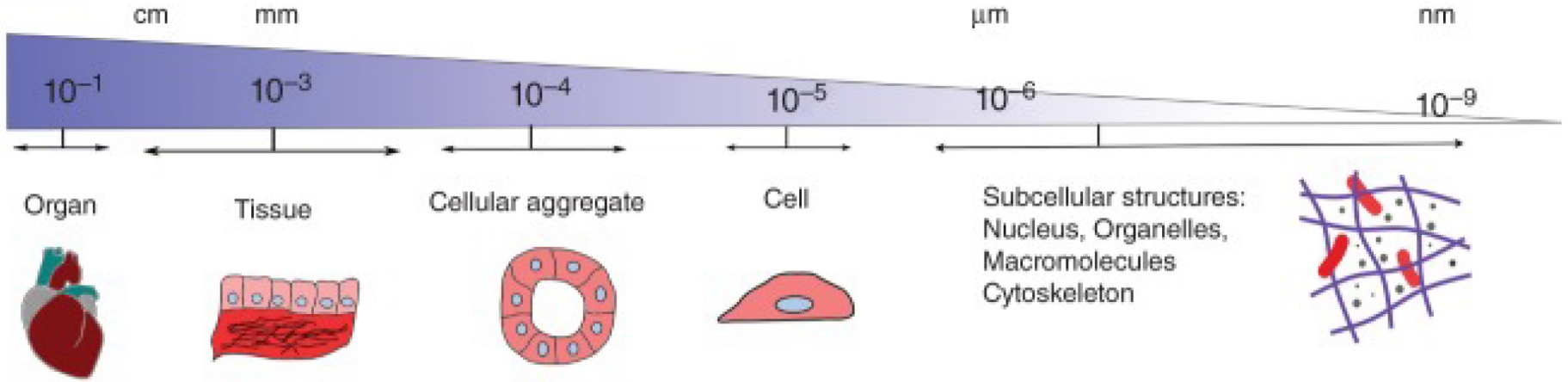
Translation

Regulation

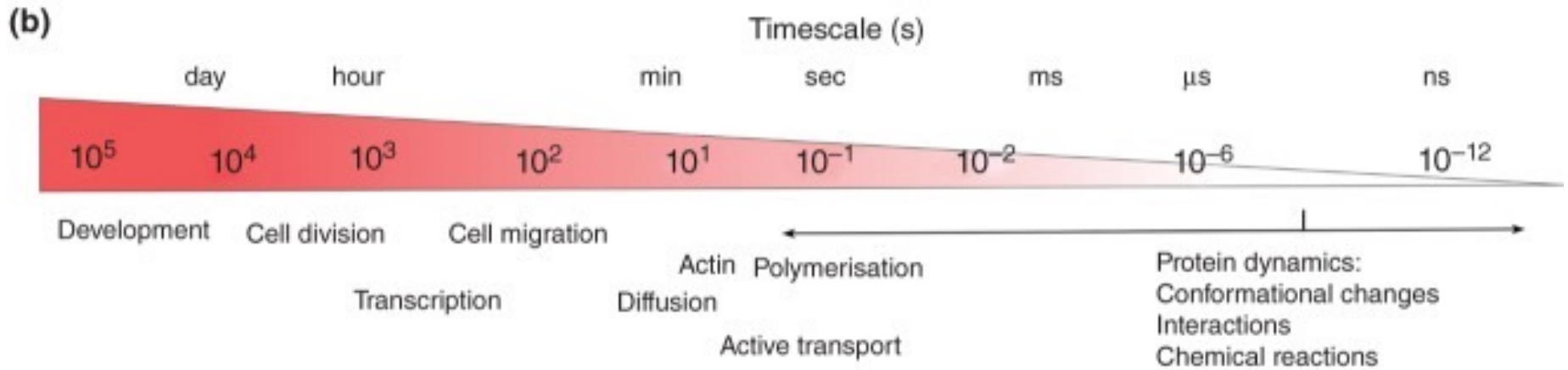
Conclusion



Length scales

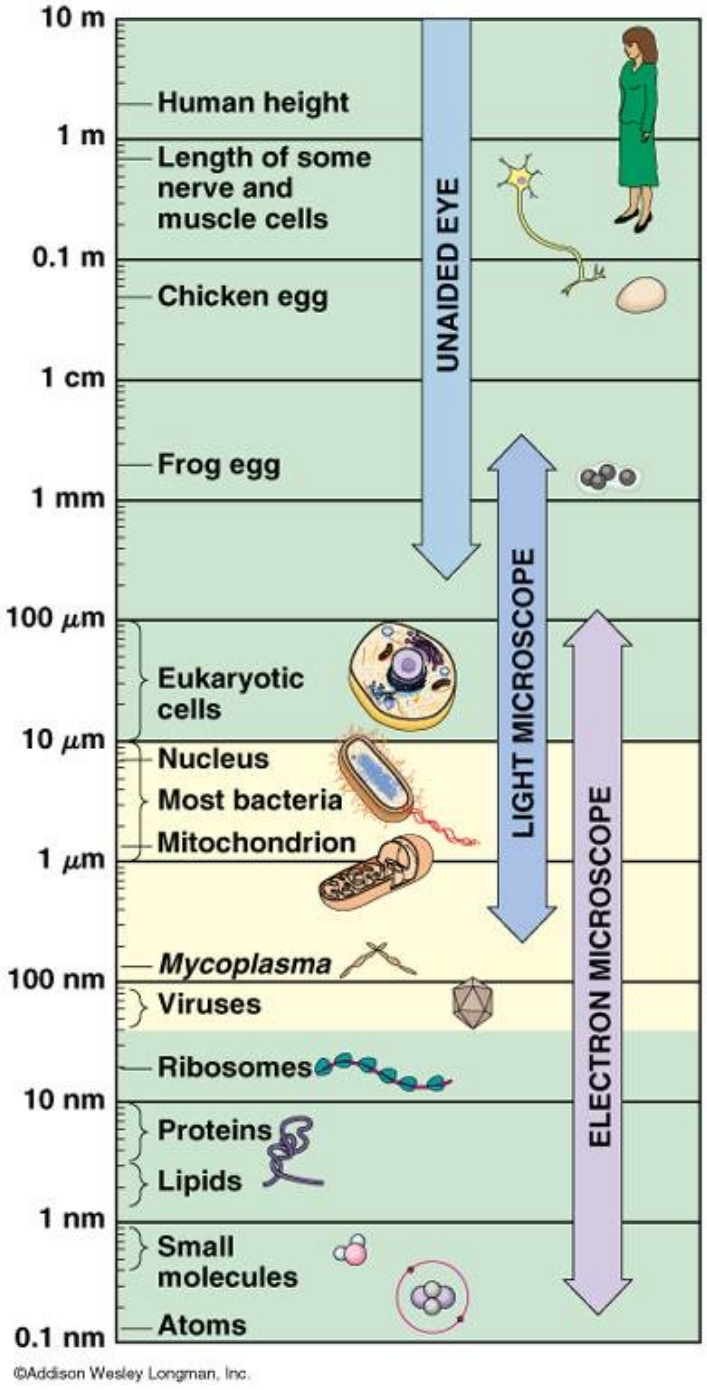


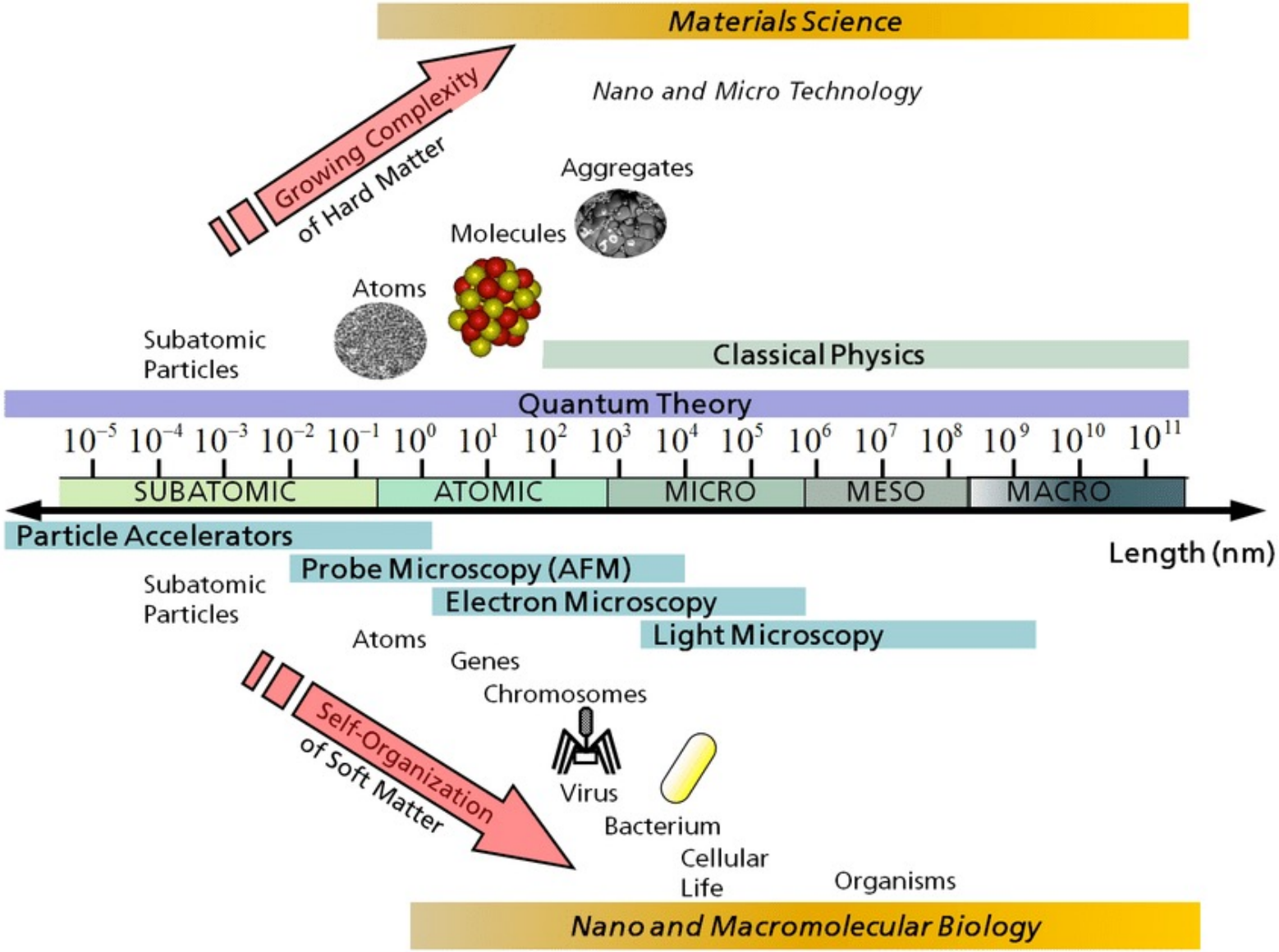
Time scales



Moeendarbary, E.; Cell mechanics: Principles, practices, and prospects. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **2014**, *6*, 371–388.

Microscopy



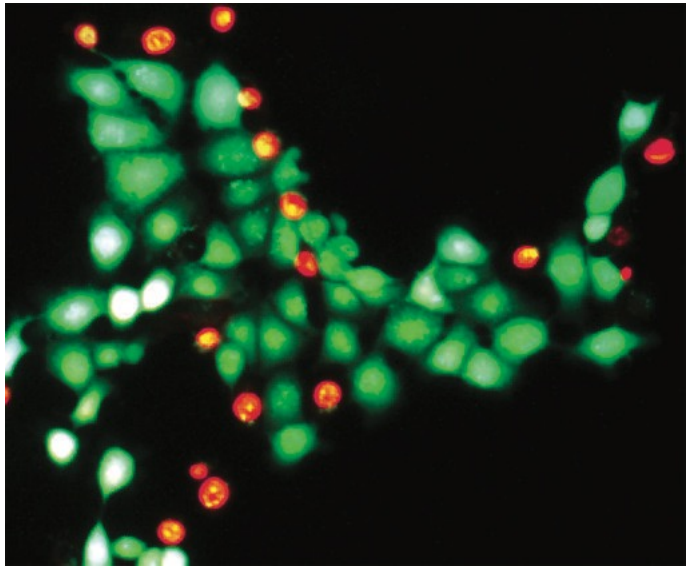


https://onlinelibrary.wiley.com/doi/pdf/10.1002/wsbm.1275

What to characterize in hydrogels?

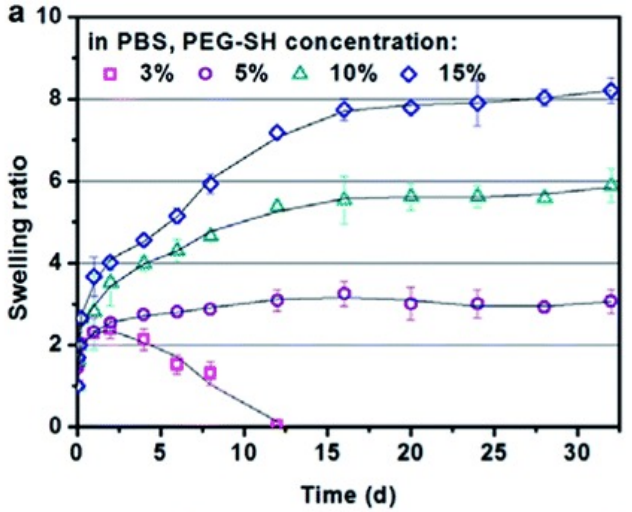
Stiffness

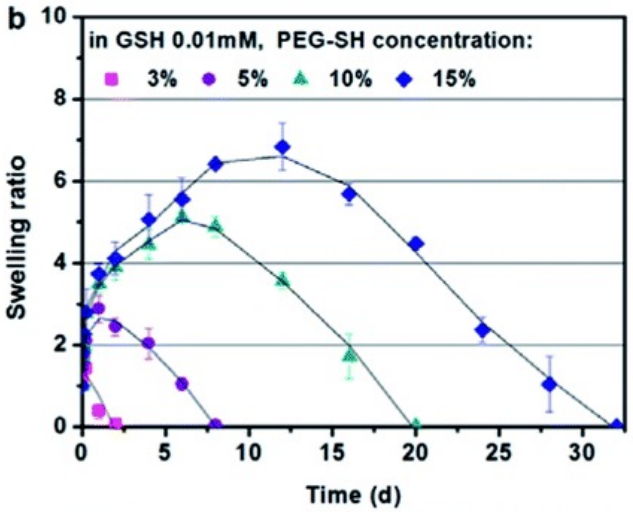
Biocompatibility

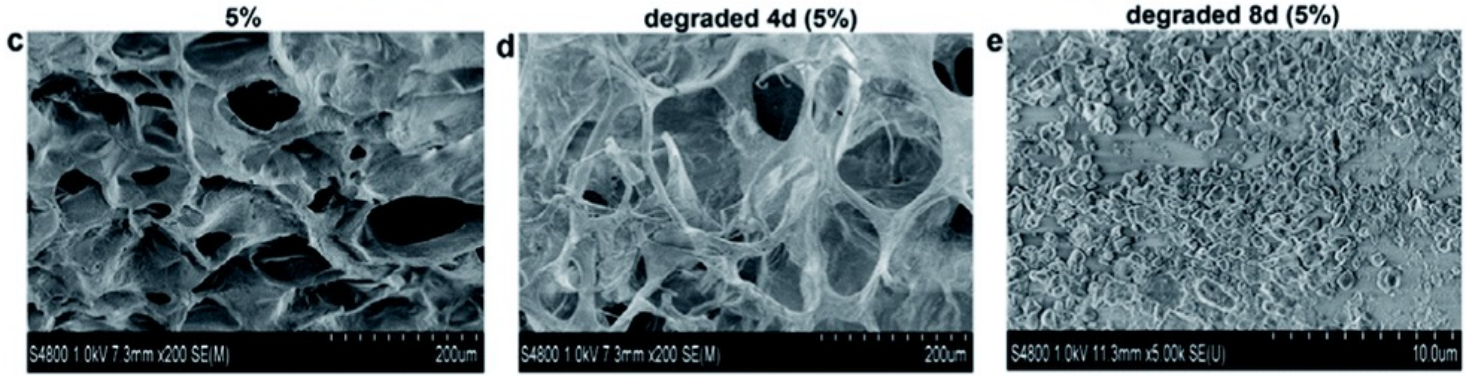


Degradation

Swelling







How to measure stiffness?

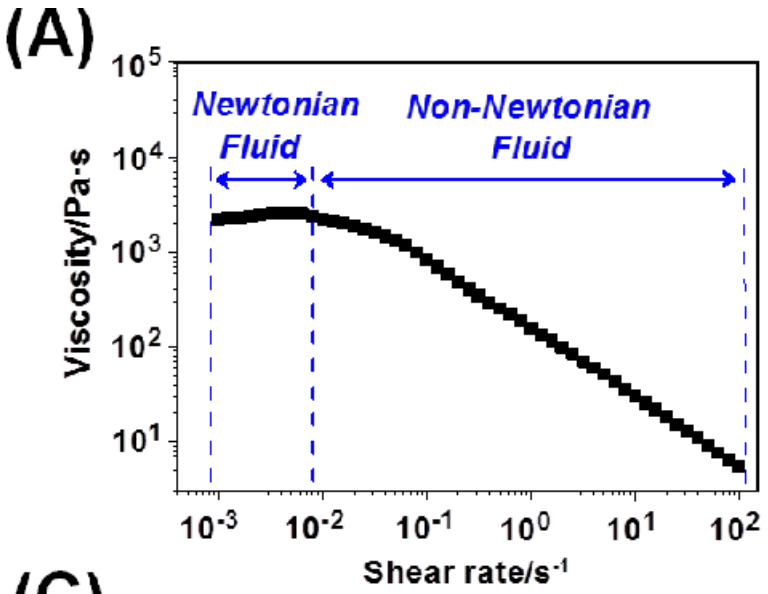
RHEOLOGY

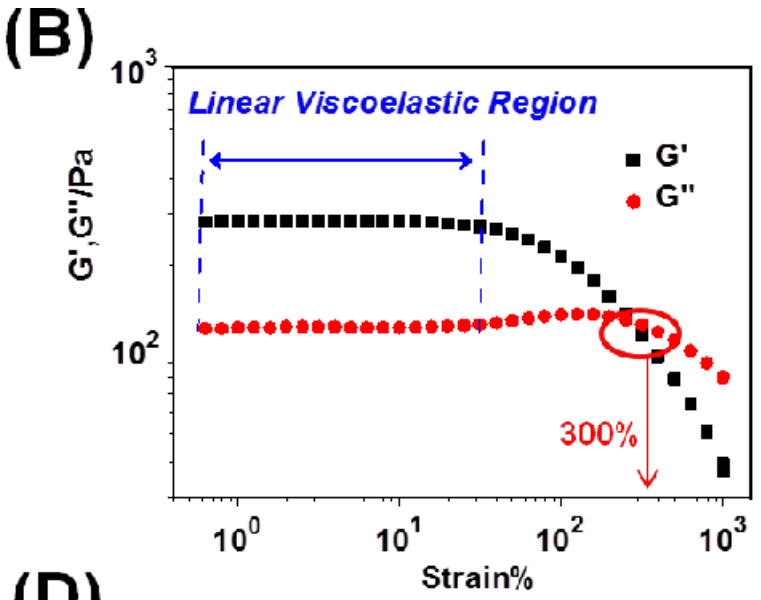
(A) Flow sweep was performed at 25 C with shear rate varying from 0.001 to 100 s -1 .

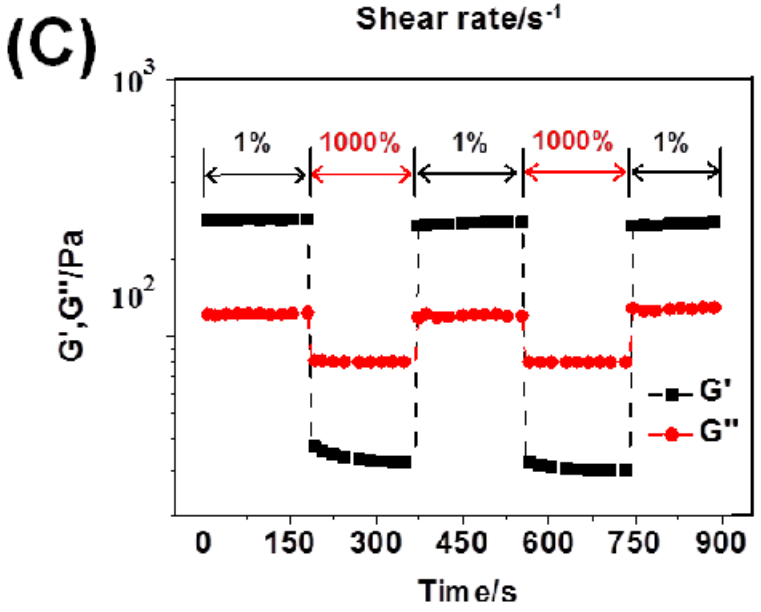
(B) Rheological strain sweep was performed from 0.5 to 1000% at 25 C with a fixed frequency of 1 Hz.

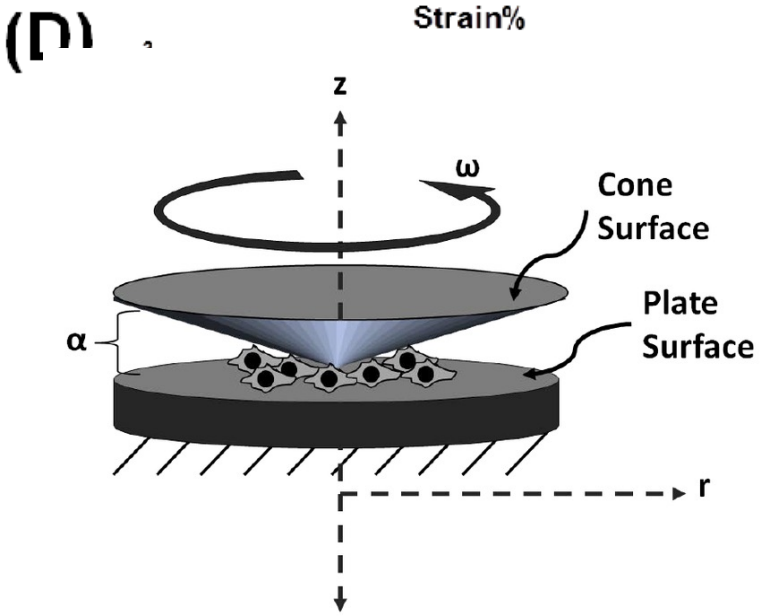
(C) Time scan tests were performed with an alternating strain of 1 and 1000% with a fixed frequency (1 Hz) at 25 C for 3 min.

(D) Rheology cone-plate setup





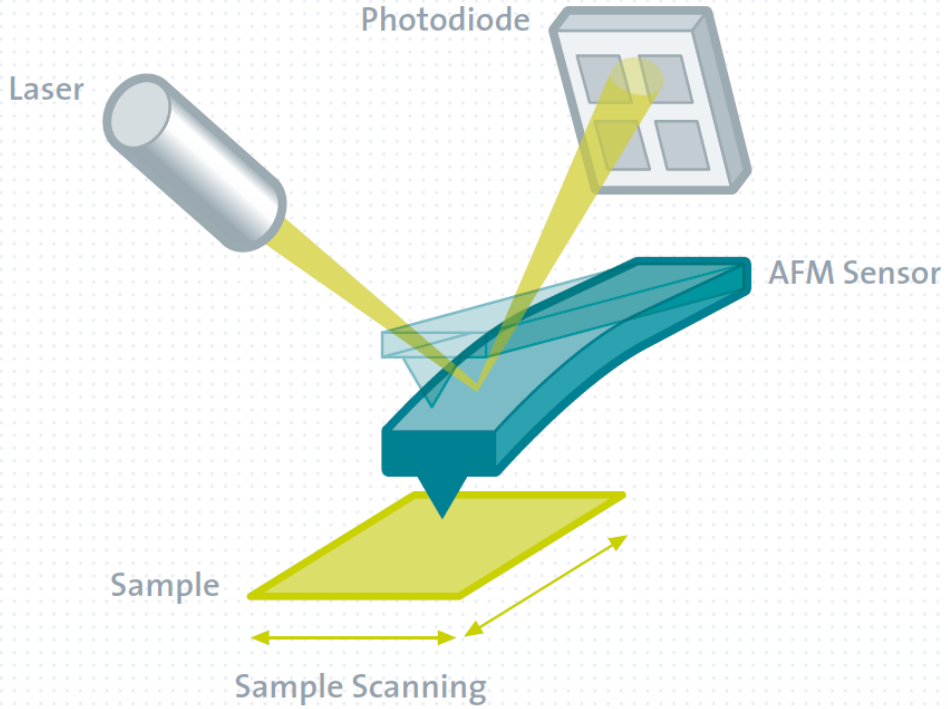


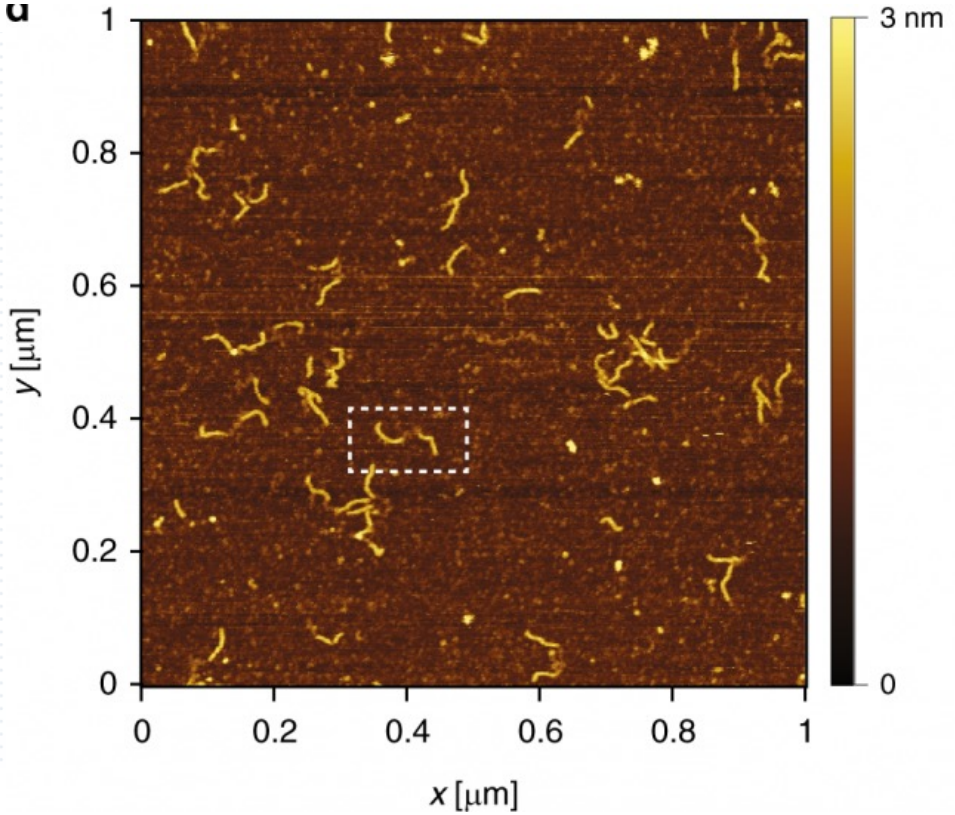


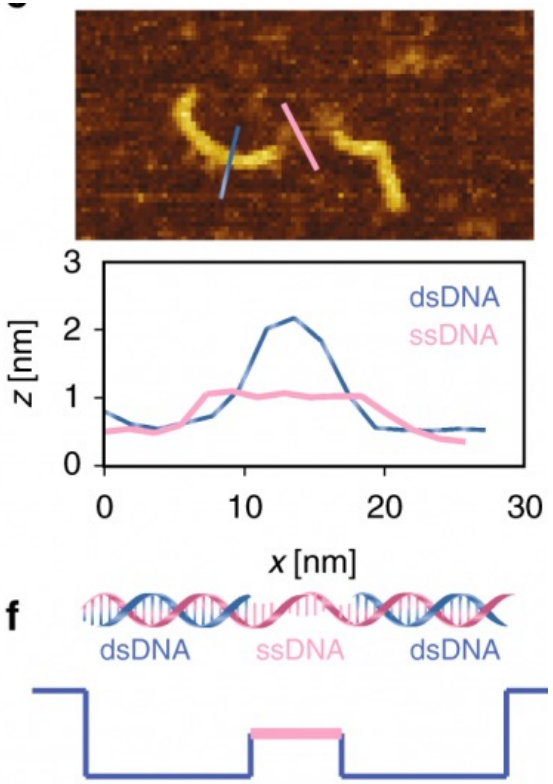
Advanced materials 2015.
Chuang Li, Matthew J. Rowland,

How to measure stiffness?

AFM



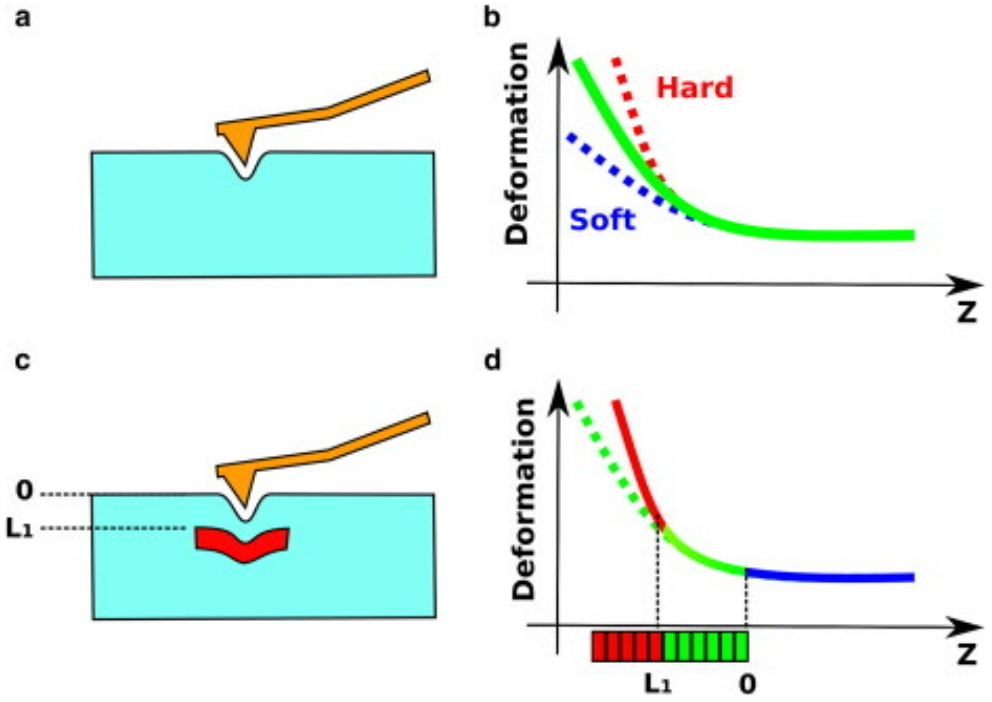


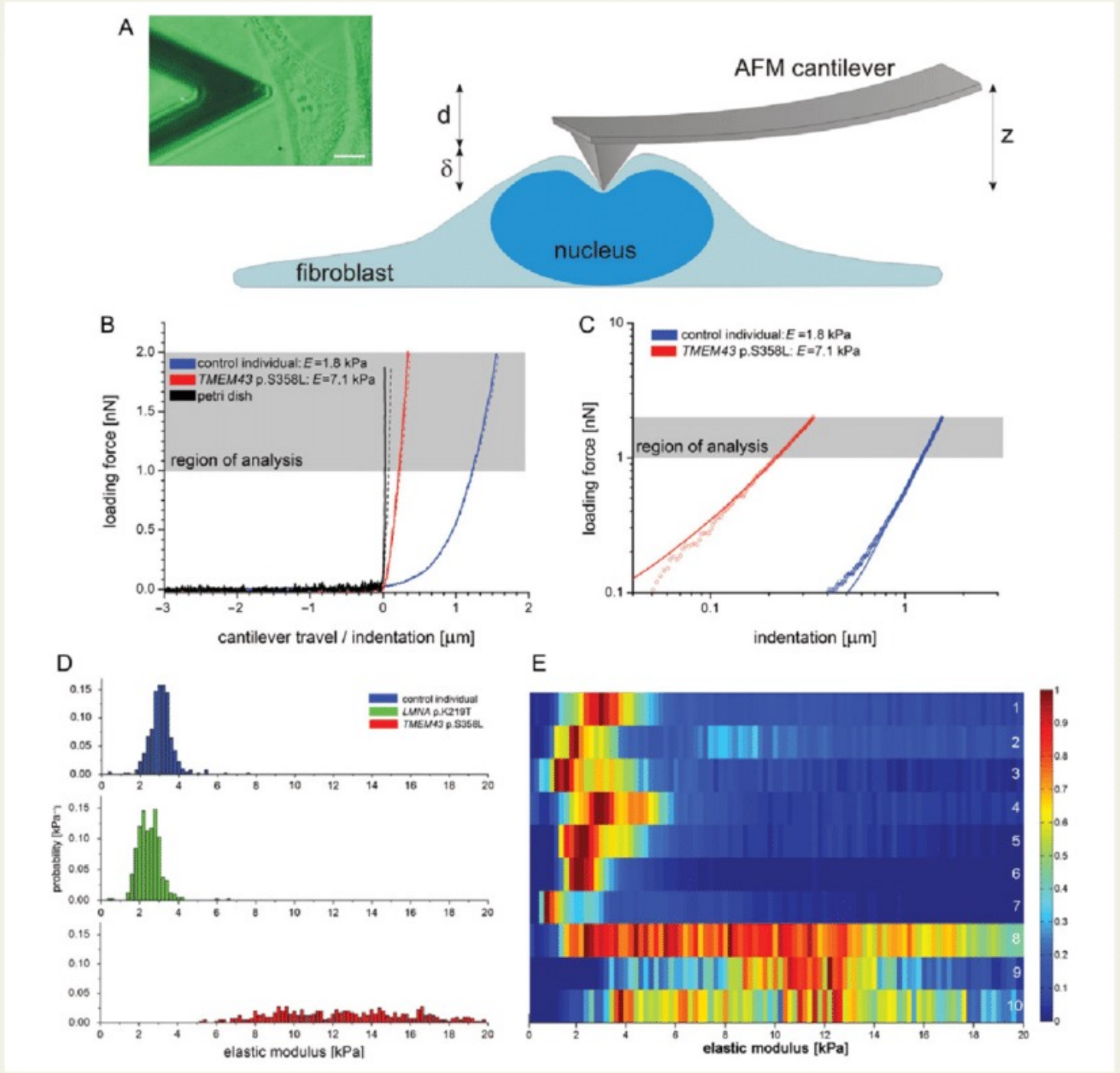


How to measure stiffness?

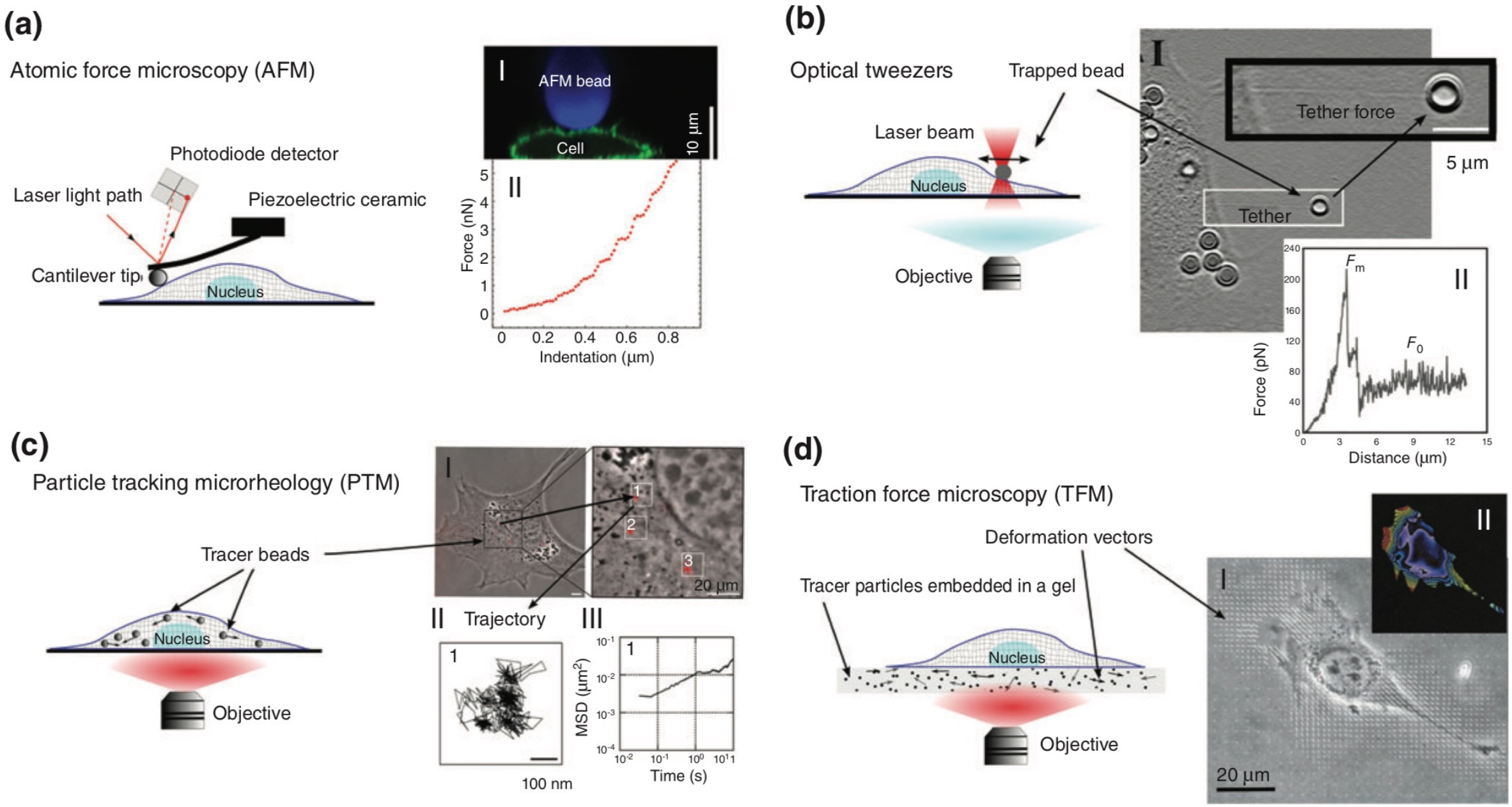
AFM

Height profiles and indentation variation



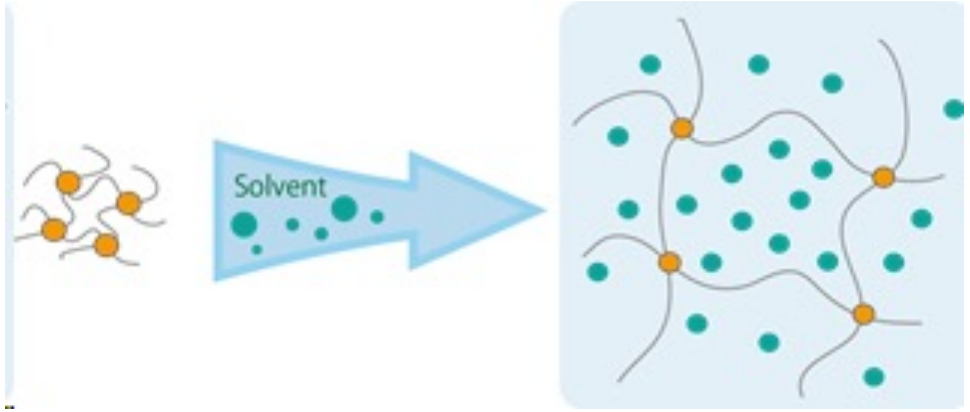


Measuring stiffness with microscopy



How to measure swelling?

Swelling rate is one of the most important properties of **hydrogels**. To **measure** the **swelling** rate, the profile of **swelling** capacity versus time of a **hydrogel** sample is obtained by performing absorbency capacity **measurements** at consecutive time intervals.



The degree of swelling can be calculated as the following:

Degree of swelling = [(Wet weight – Dry weight) / Dry weight] ×100%

The water content of hydrogels is calculated after the equilibrium swelling by

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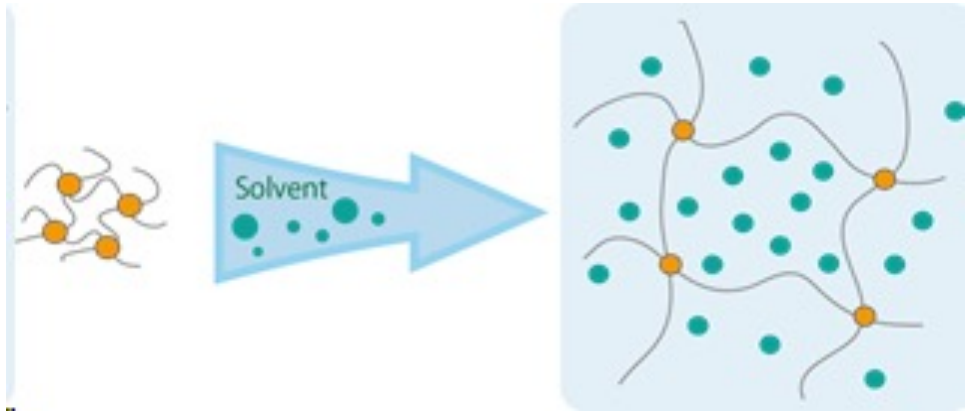

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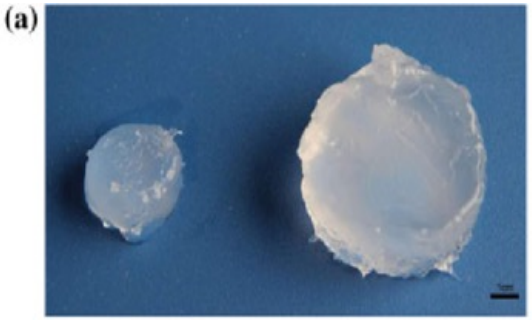

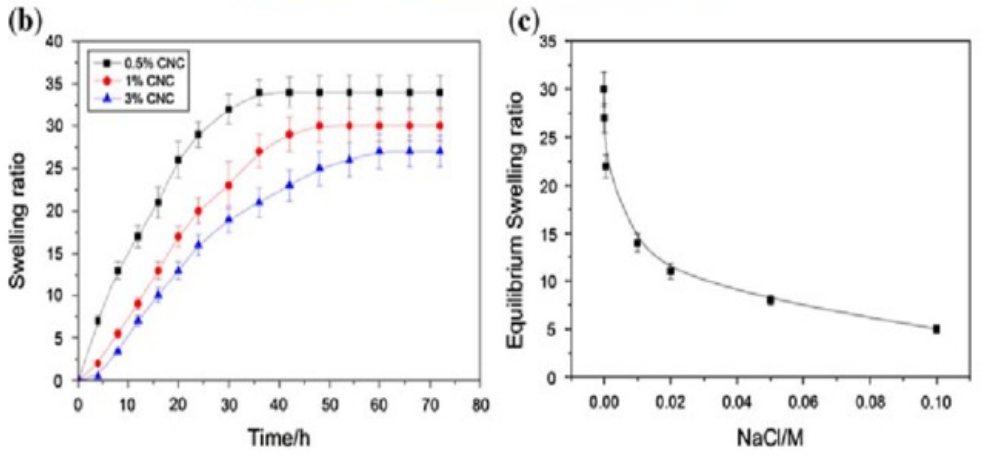

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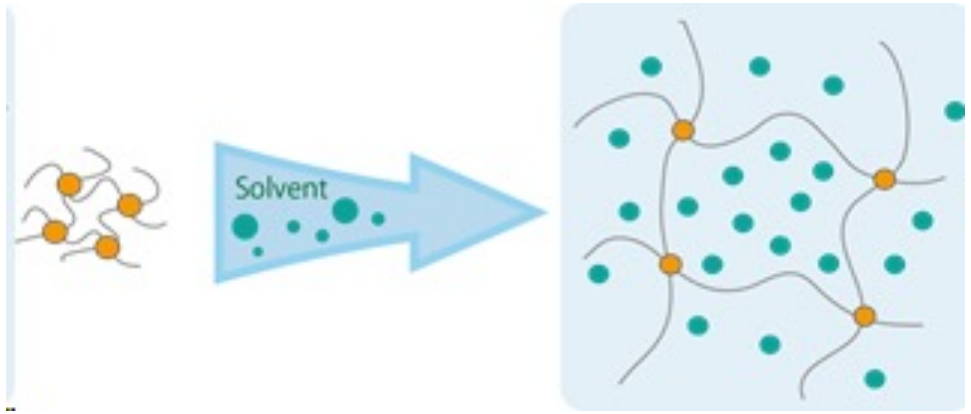

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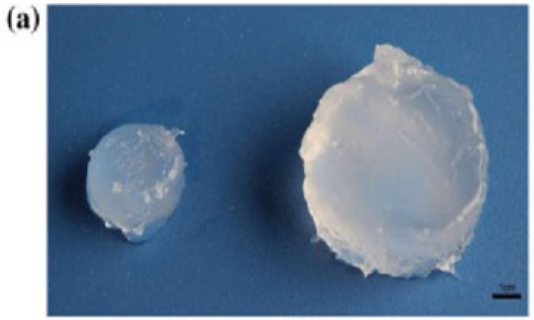
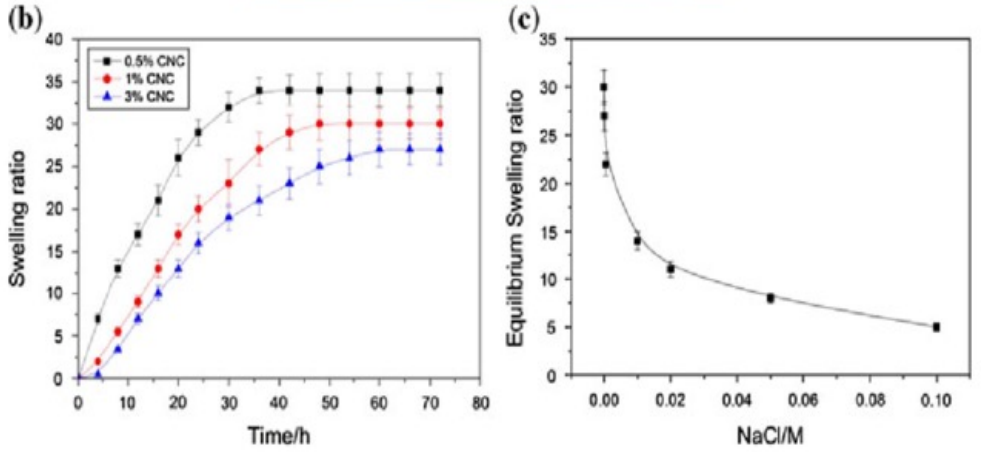


Because water acts as a plasticizer in a hydrophilic polymer network system, the swelling process can be described by the free energy of mixing ΔG_{mix} from the polymer and solvent interaction and the elastic free energy $\Delta G_{elastic}$ from the crosslinked network: $\mathbf{\Delta G_{system} = \Delta G_{mix} + \Delta G_{elastic}}$
During the processing of swelling, the ΔG_{mix} and $\Delta G_{elastic}$ both increased until $|\Delta G_{mix}| = |\Delta G_{elastic}|$ and $\mathbf{\Delta G_{system} = \Delta G_{mix} + \Delta G_{elastic} = 0}$, so that the driving force for swelling is gone: **equilibrium swelling is reached and swelling stops**.



How to measure degradation?

In vitro degradation

Solutions with different combinations of chitosan and Gp salt were placed into circular-shaped molds (diameter 4 mm) and allowed to make a gel in an incubator at 37°C. After 10 h, the gels were removed and suspended in 10 mL of isotonic phosphate buffered saline (PBS, pH=7.4) containing 4 mg mL^{-1} lyzozyme. The samples were placed in a shaking incubator at 37°C and 50 rpm. After predetermined intervals of time, samples were removed from the medium, rinsed with distilled water, dried under vacuum and collected for the analysis.

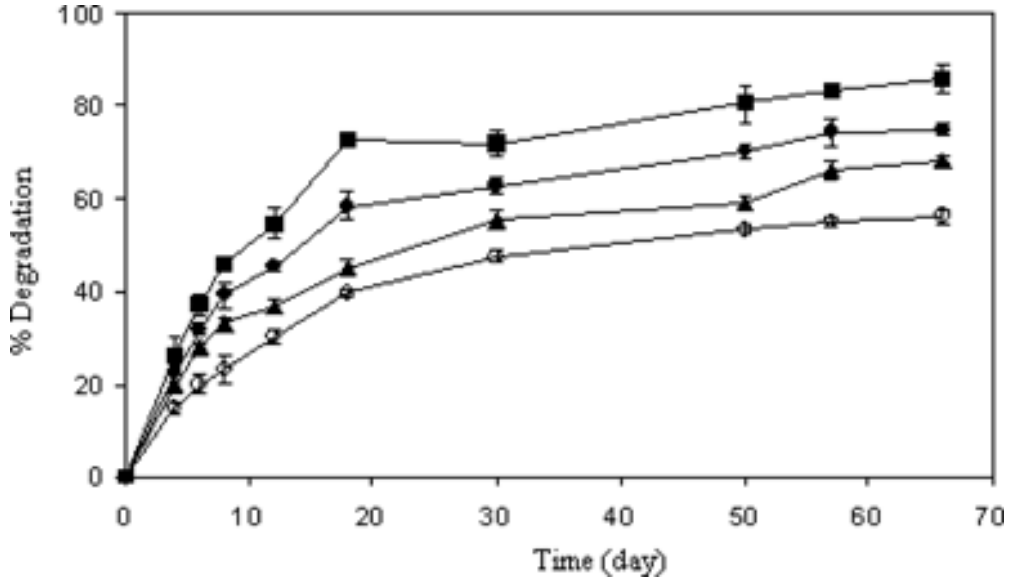
Degradation was quantified using the following equation:

$$\%Degradation(t)=[Wd(0)-Wd(t)]/Wd(0)$$

where $W_{d(0)}$ is the initial dry polymer mass and $W_{d(t)}$ is the dry polymer mass at time t.

In-Vivo Gel Degradation Experiment

Hydrogel disks (6 mm diameter) were subcutaneously implanted into the backs of male 4–5 week old anesthetized CB-17 SCID mice (n = 4/time point condition). Hydrogel disks were harvested after 2, 4, and 14 weeks, mechanically tested, and processed for histological analysis by paraffin embedding, sectioning, and staining with hematoxylin and eosin.



https://doi.org/10.1007/s10971-006-9007-1

10.1073/pnas.0737381100

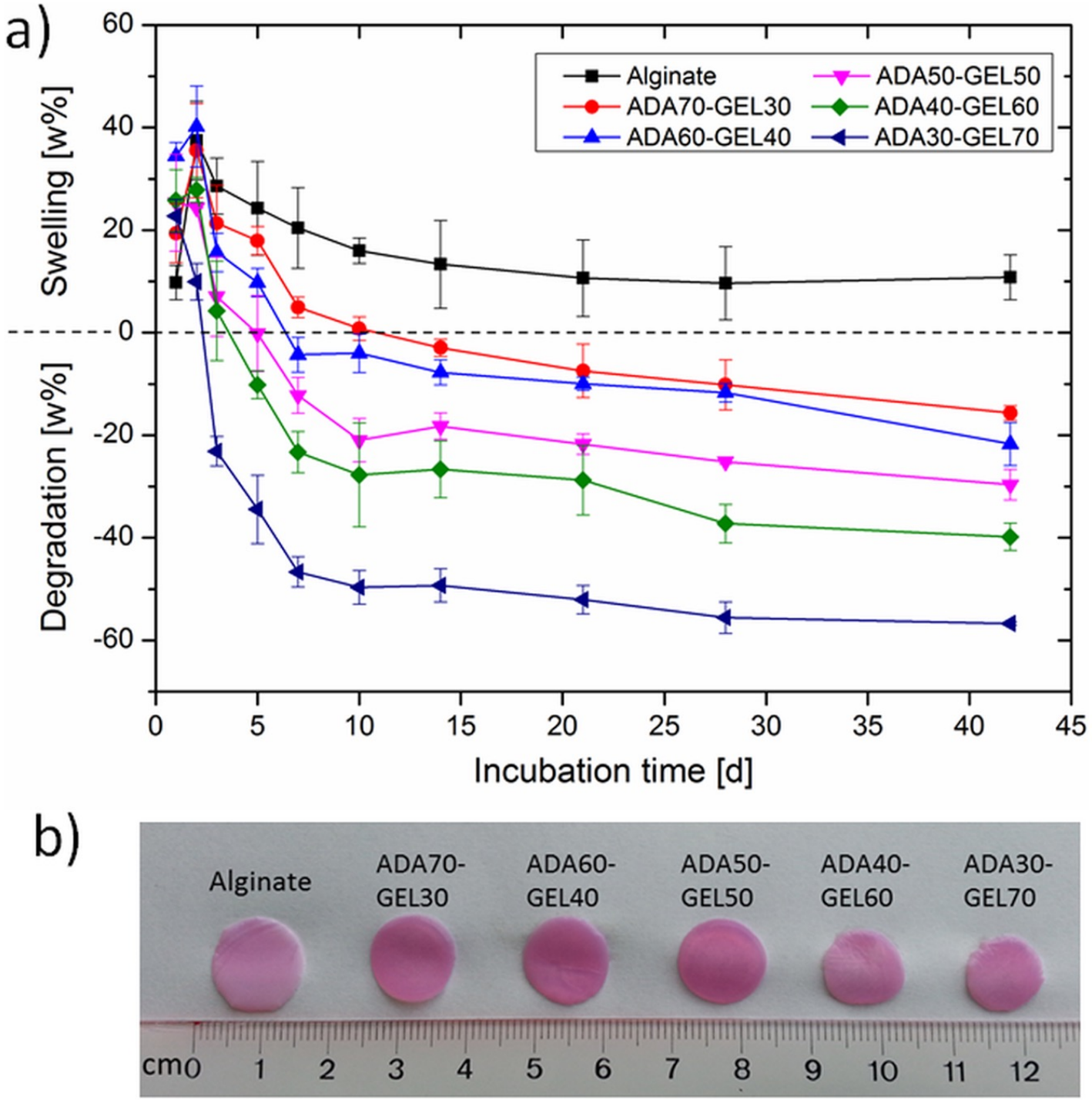


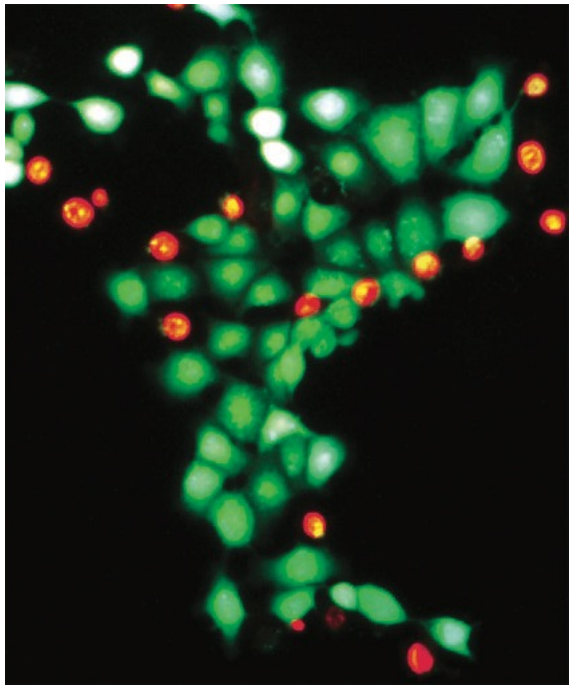
Figure 2. Swelling and degradation characteristics of alginate and ADA-GEL hydrogels.

(a) Swelling and degradation as a function of incubation time in DMEM of the films fabricated from alginate and ADA-GEL hydrogels of different compositions and
(b) photograph of the hydrogel films after 28 days of incubation during degradation study.

https://doi.org/10.1371/journal.pone.0107952.g002

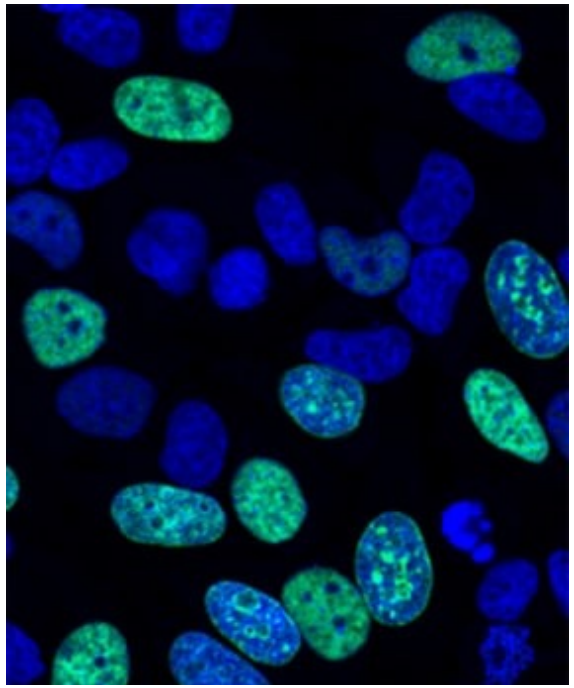
How to measure biocompatibility?

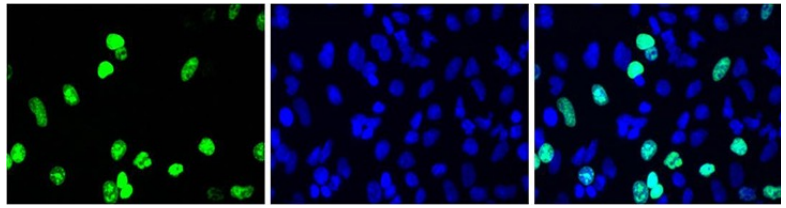
Live-dead viability assay



Detect living cells

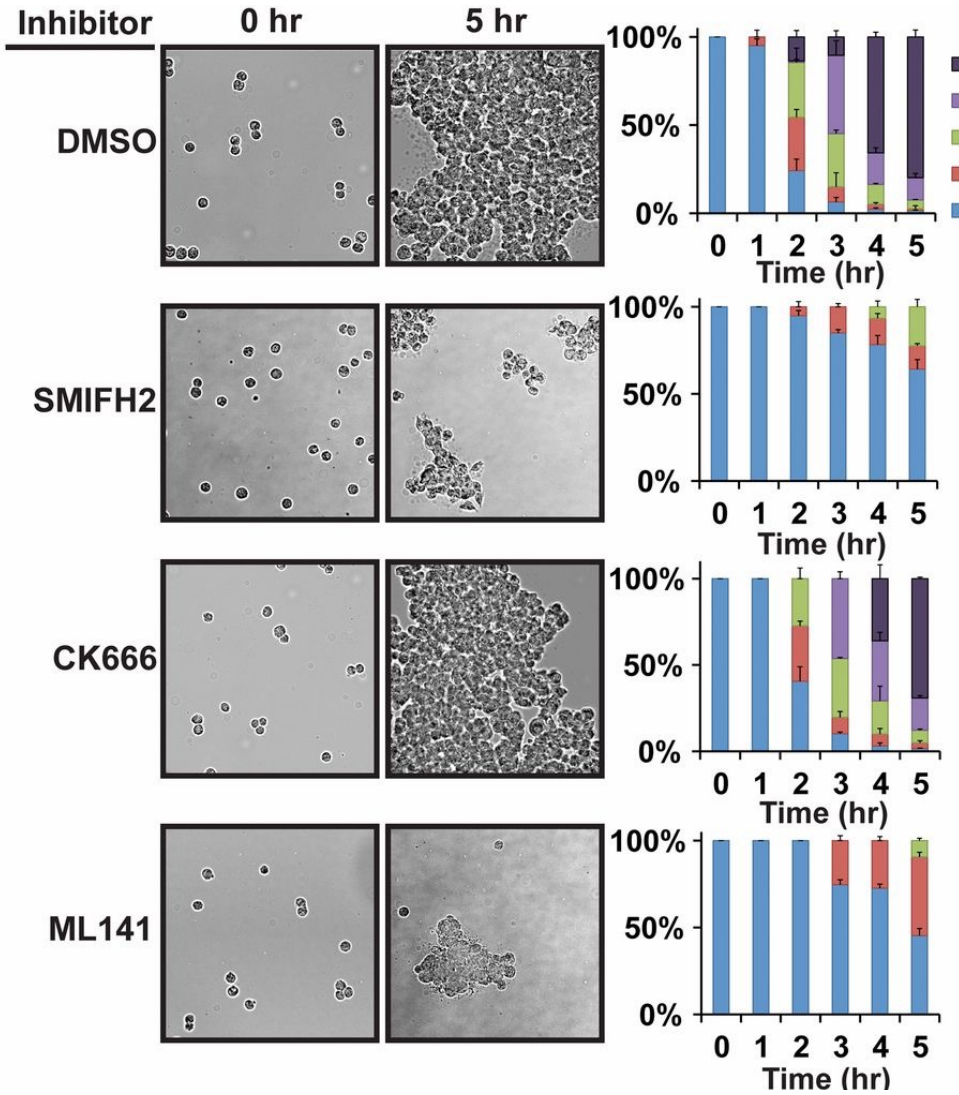
Proliferation assay





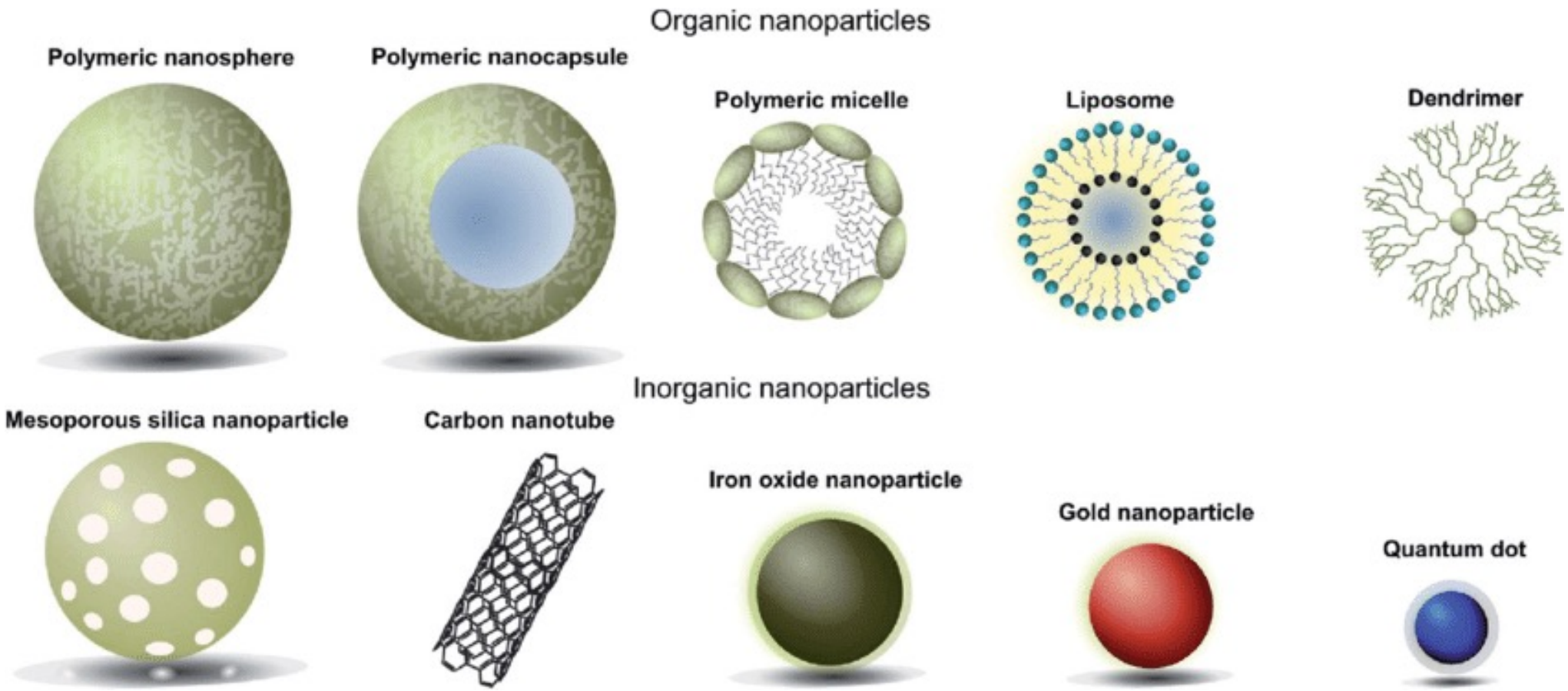
Label new synthesized DNA

Cell adhesion assay



How to characterize nanoparticles?

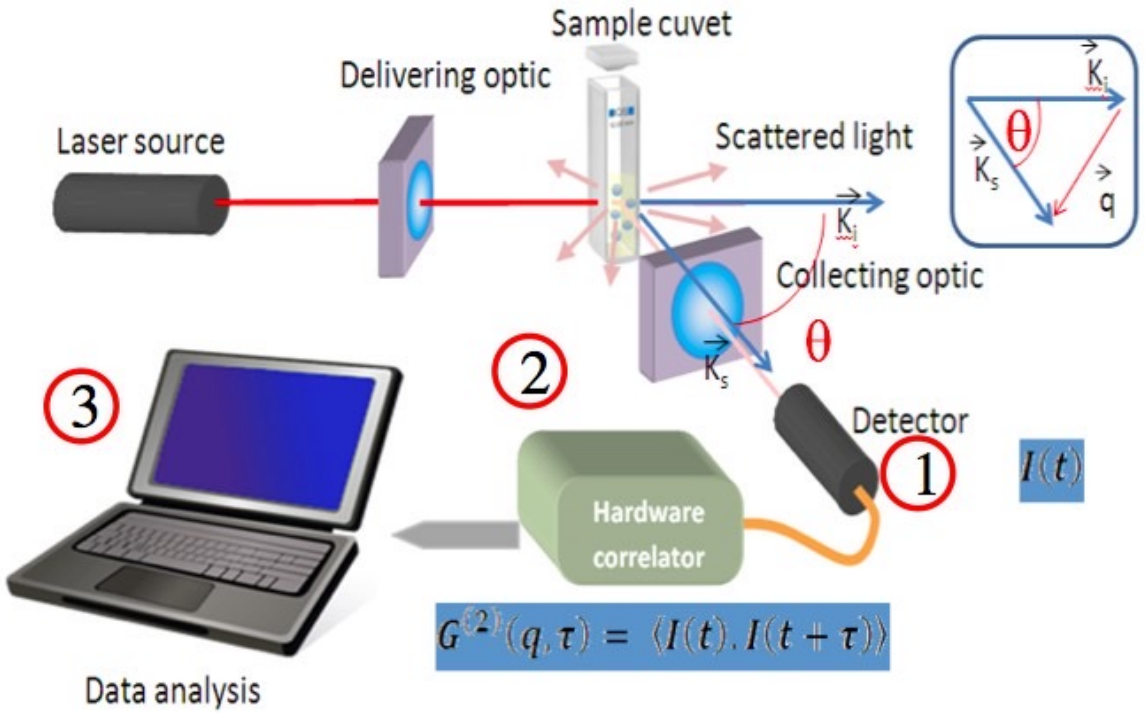
Size **Shape** **Charge** **Cell uptake** **Biodistribution**

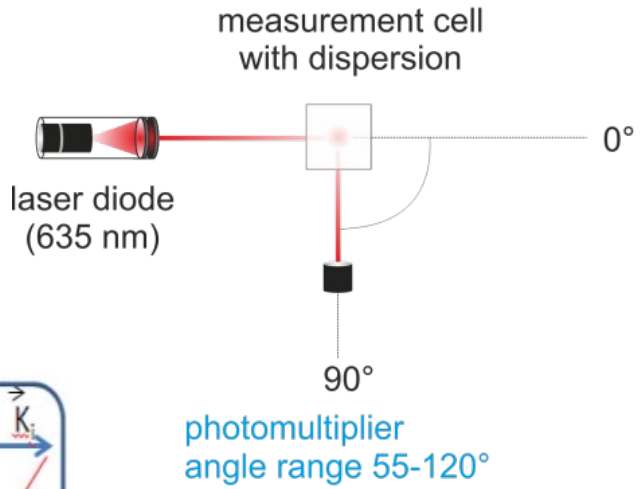


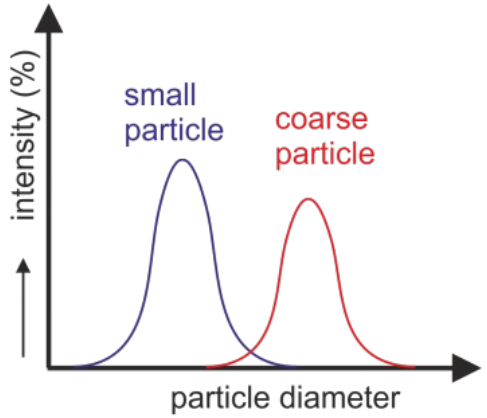
How to characterize nanoparticles?

DLS

Dynamic Light Scattering

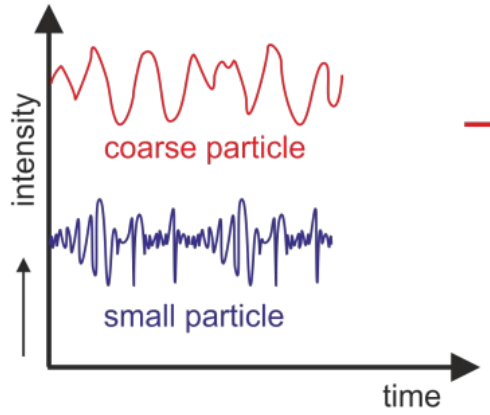


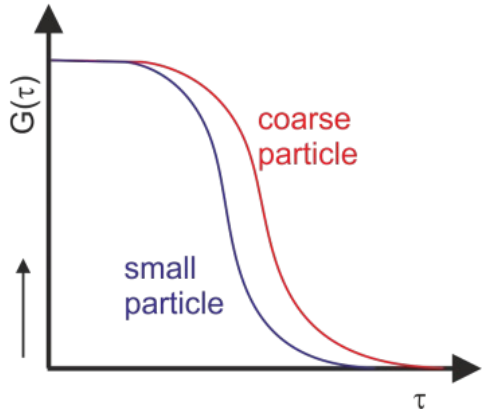




Stokes-Einstein

correlator
diffusion coefficient

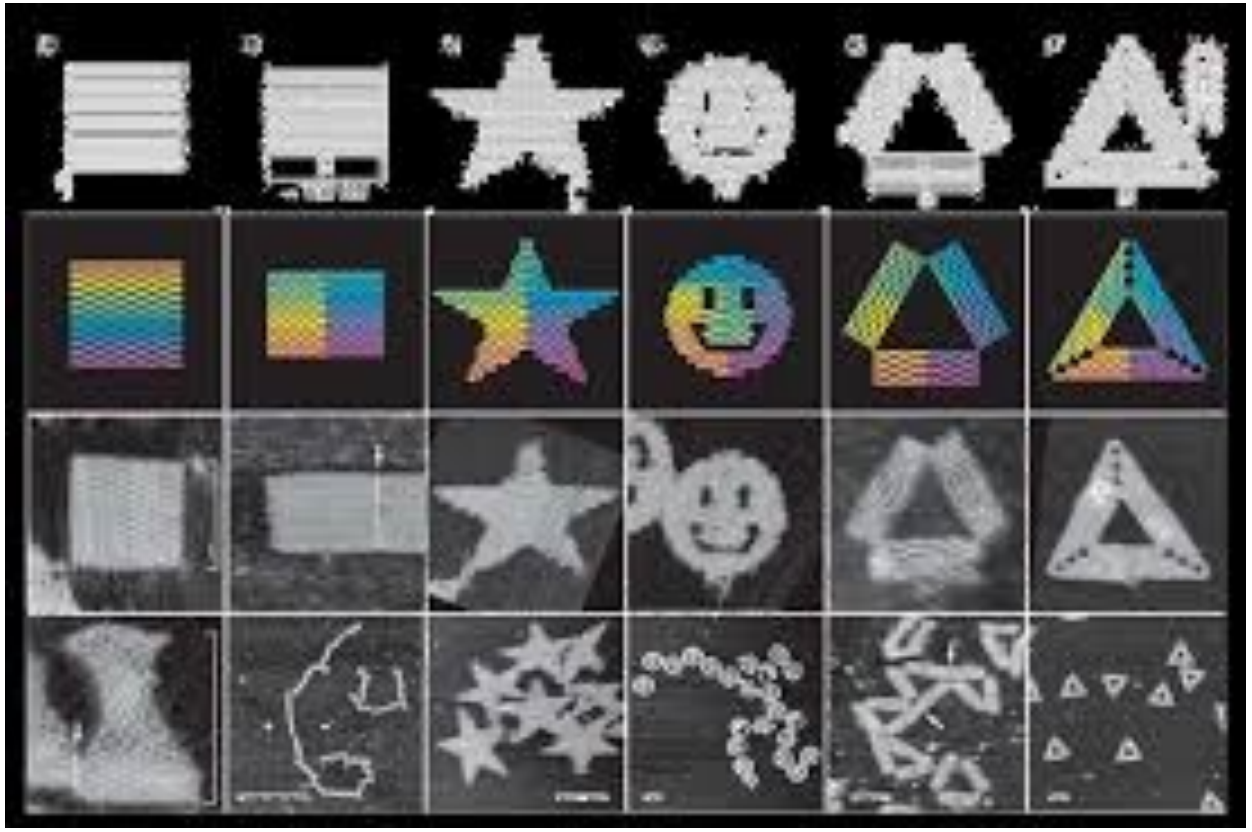




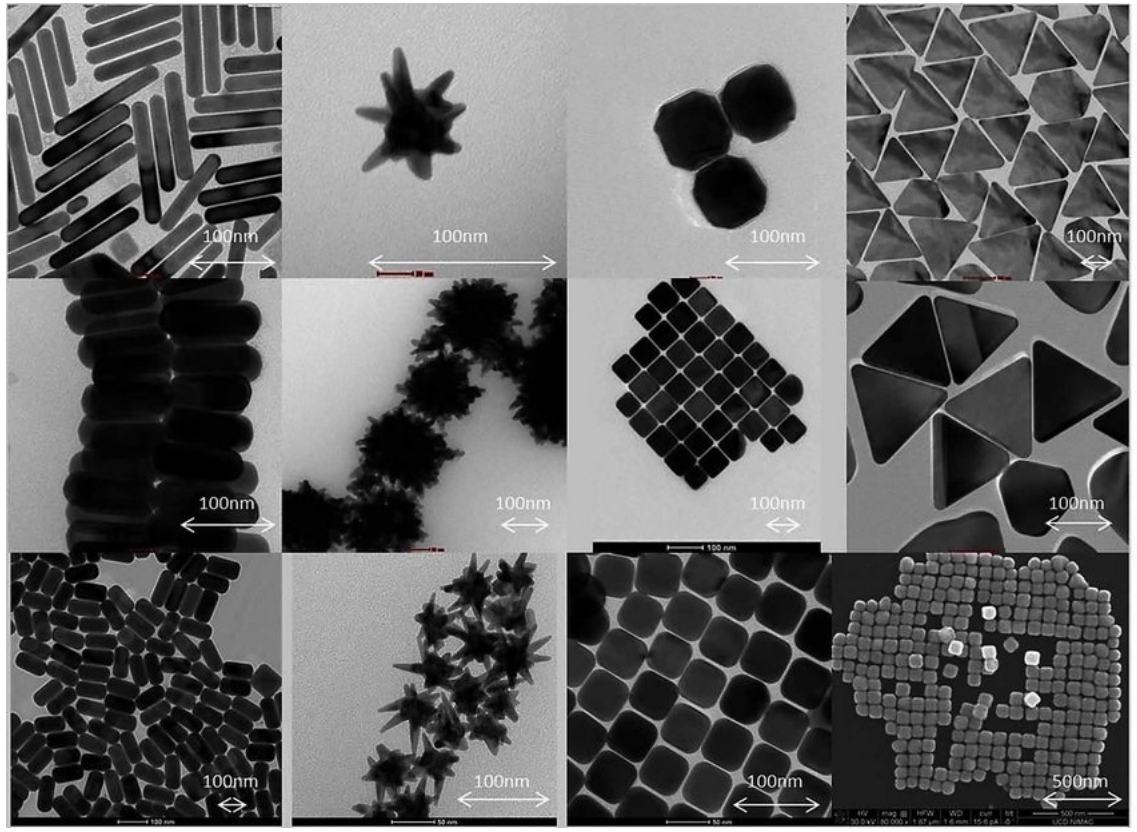
How to characterize nanoparticles?

Shape

Microscopy



AFM



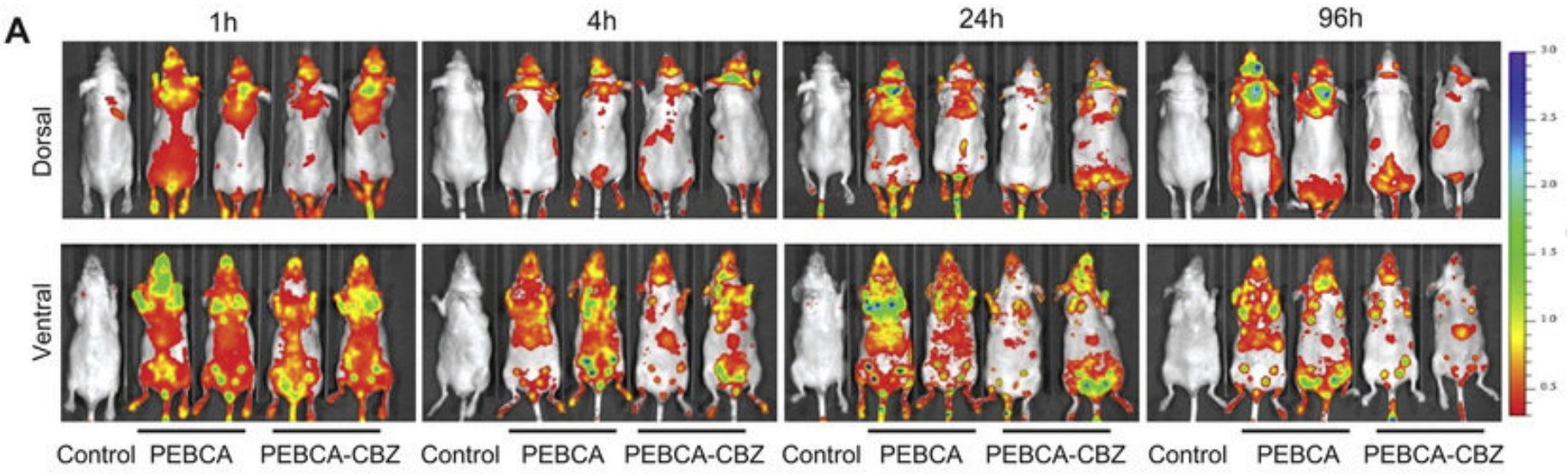
TEM. /. SEM

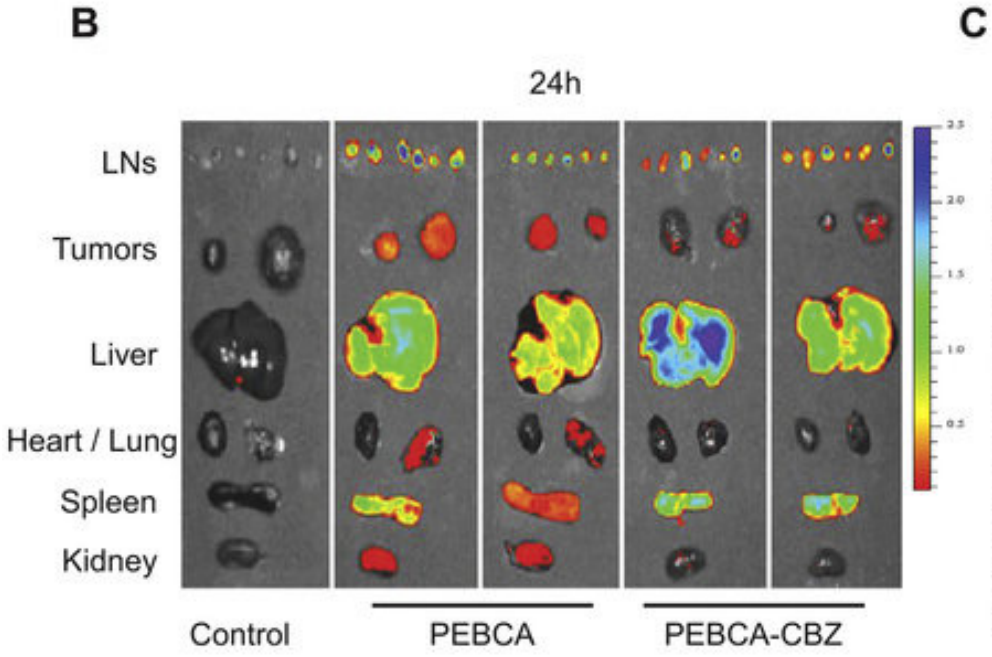
How to characterize nanoparticles?

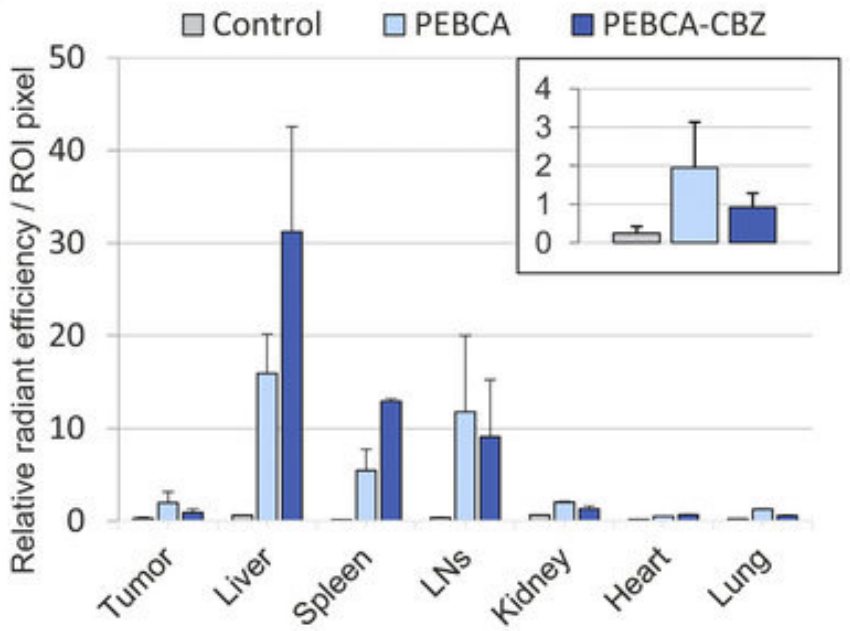
Biodistribution

IVIS

Imaging chamber



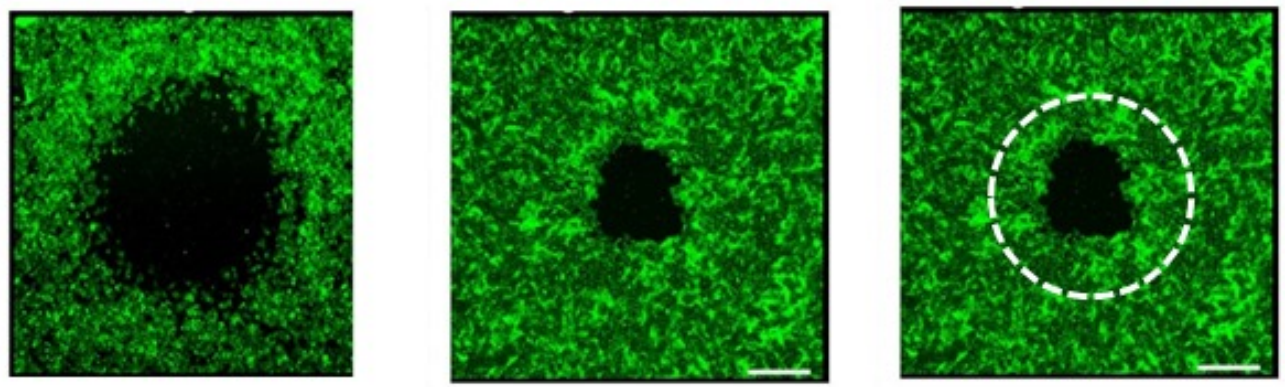


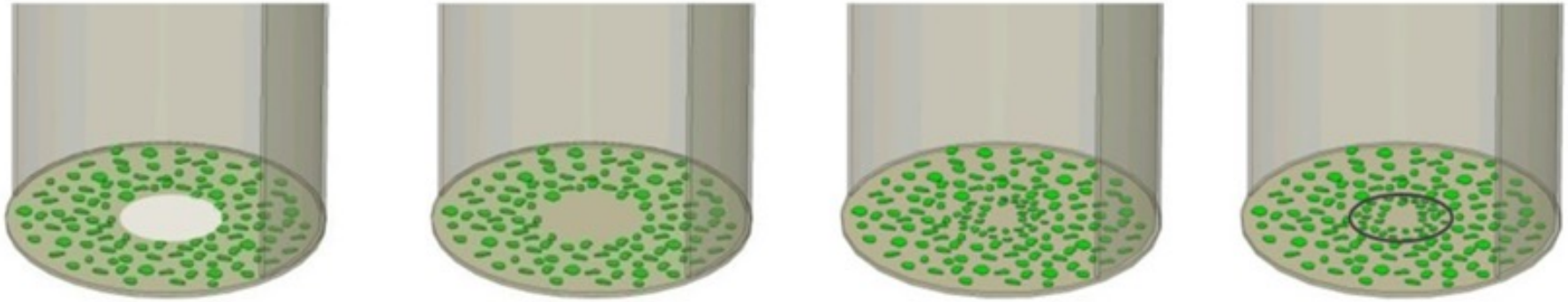


How to measure the cell material interaction?

Cell motility

Using microscopy





Seed & Adhere Cells Around BioGel

BioGel Dissolves To Create Detection Zone

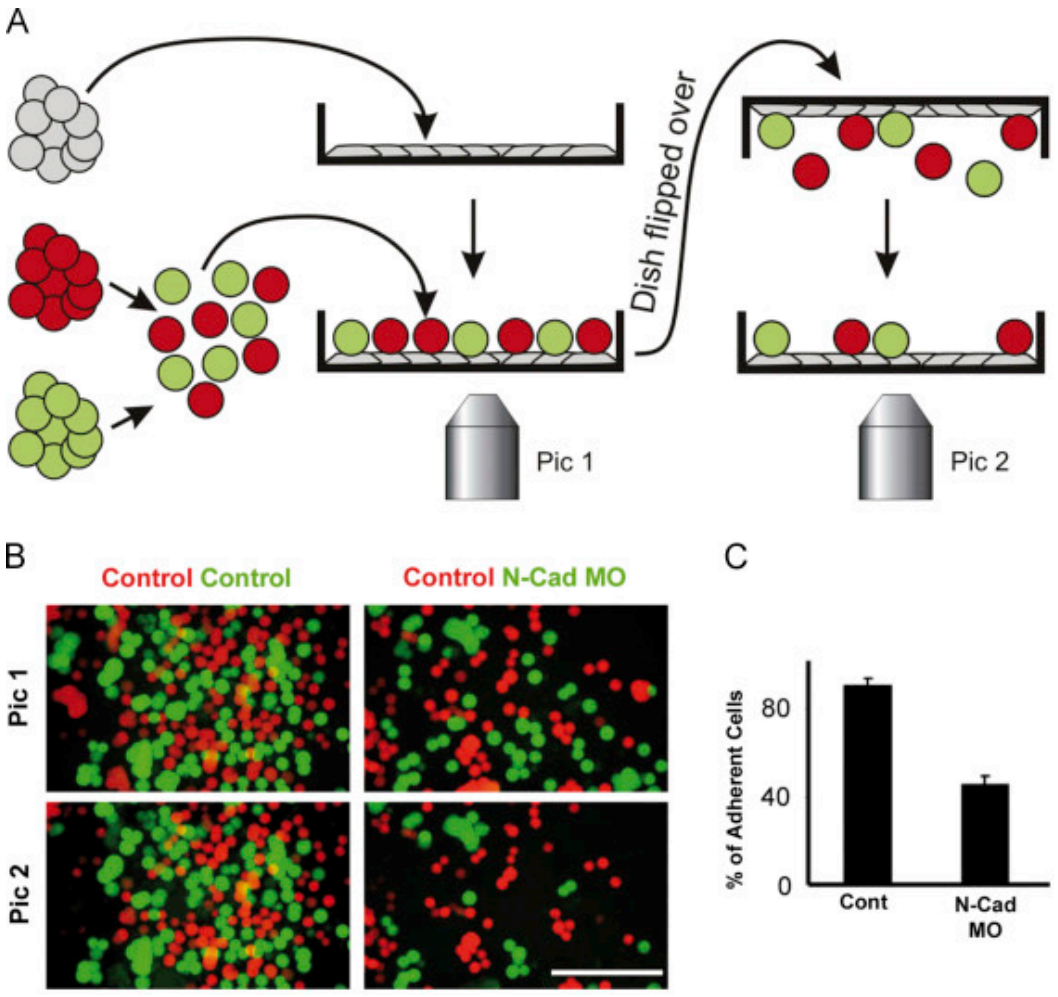
Cells Migrate into Detection Zone

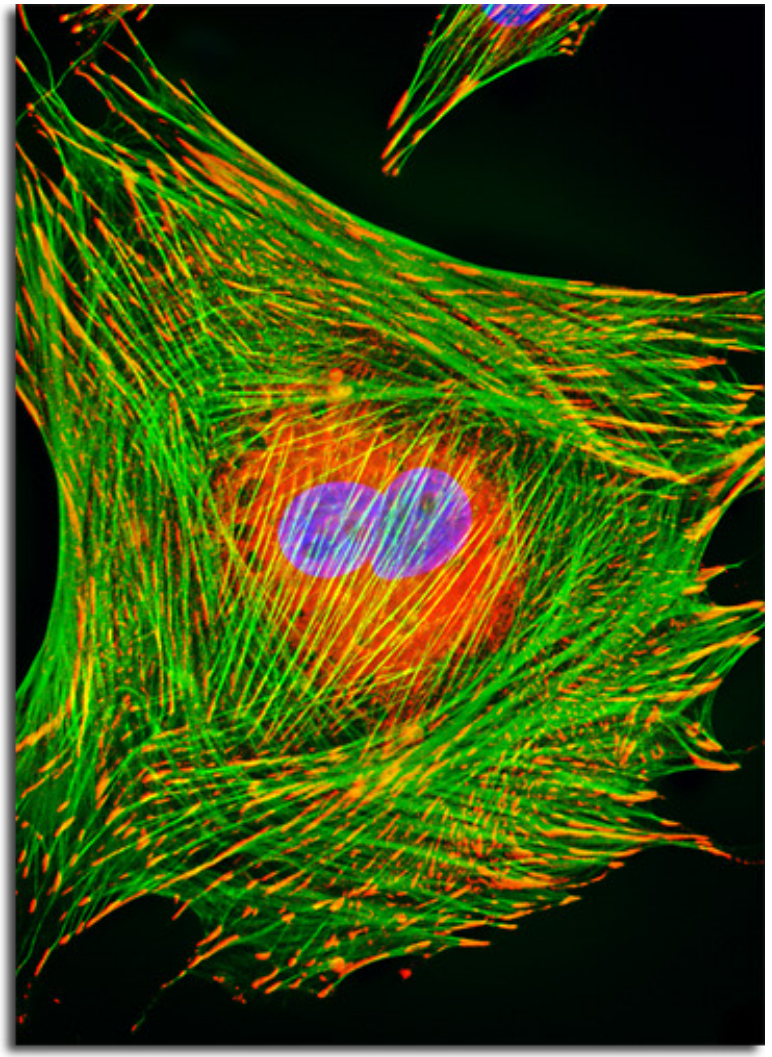
Image Cells in Detection Zone via Microscopy or HCS/HCI Instruments

How to measure the cell material interaction?

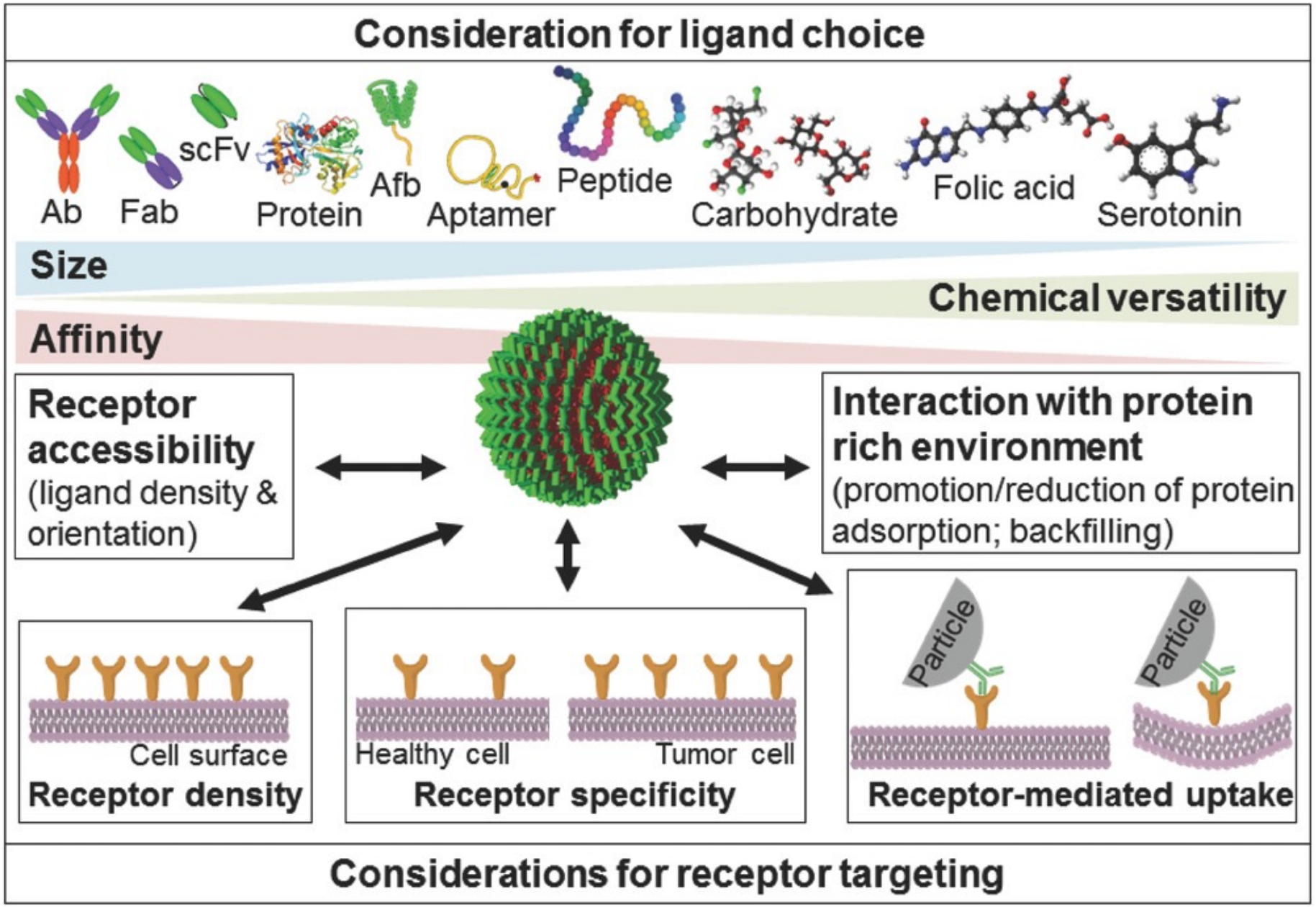
Cytoskeleton and FA

Microscopy





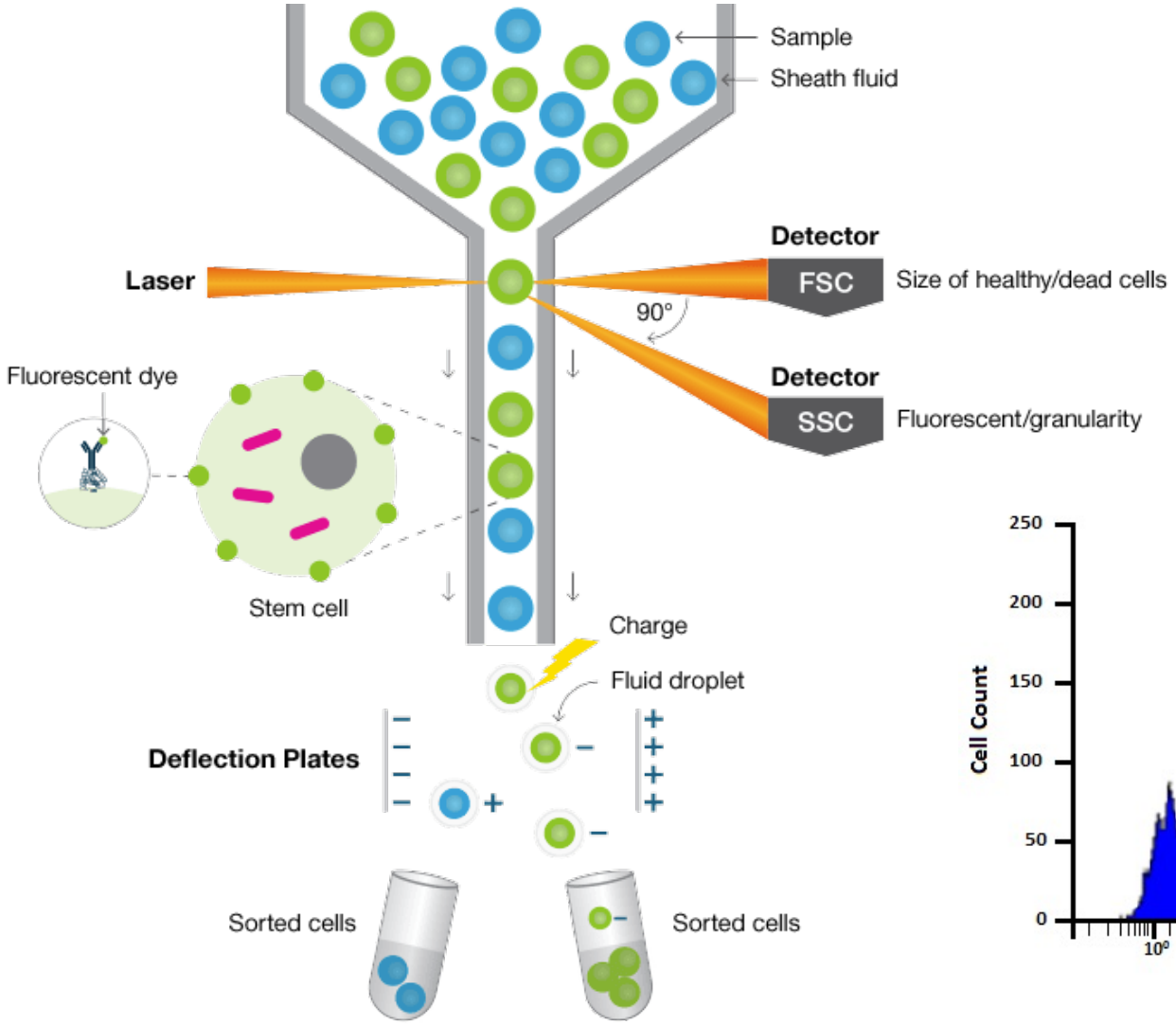
How to measure the targeting efficiency?

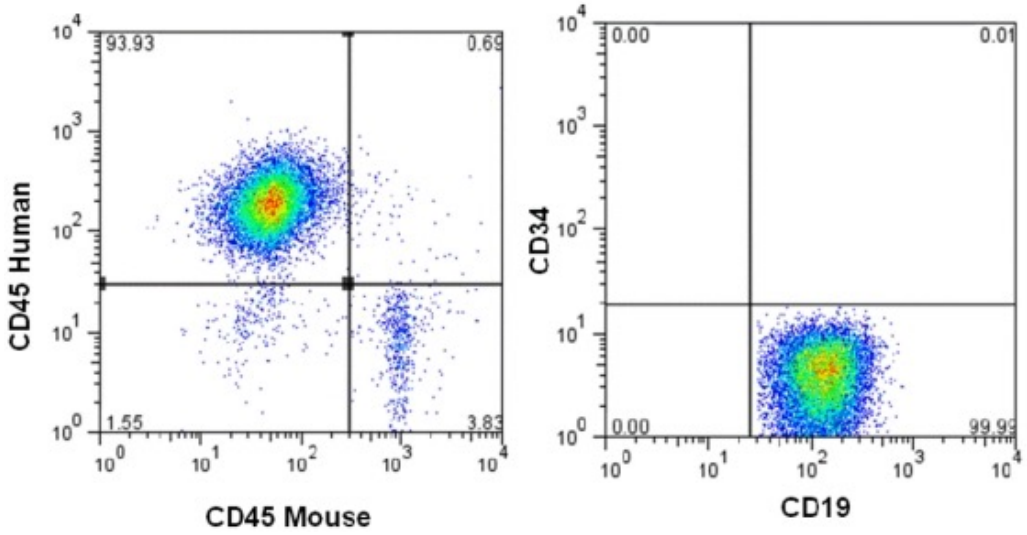


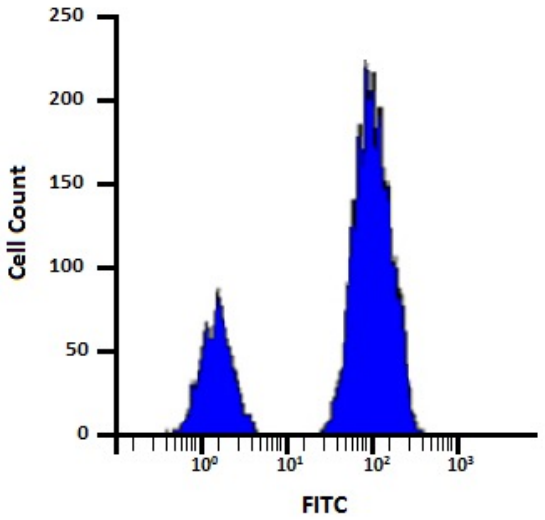
How to measure the targeting efficiency?

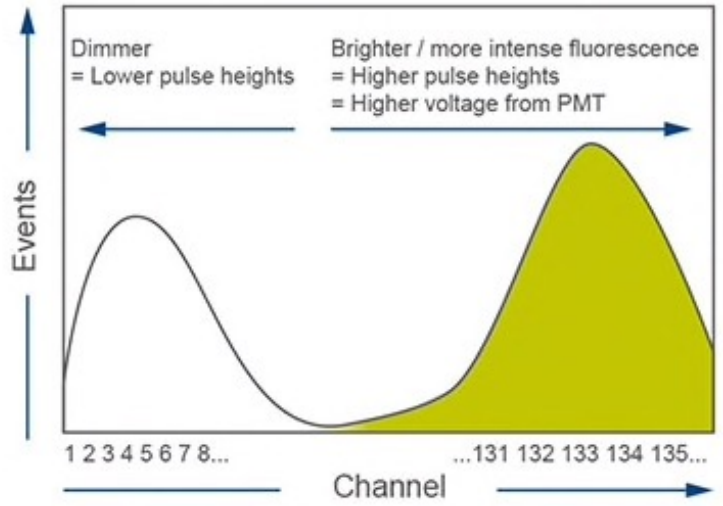
In Vitro

Flow cytometry / FACS





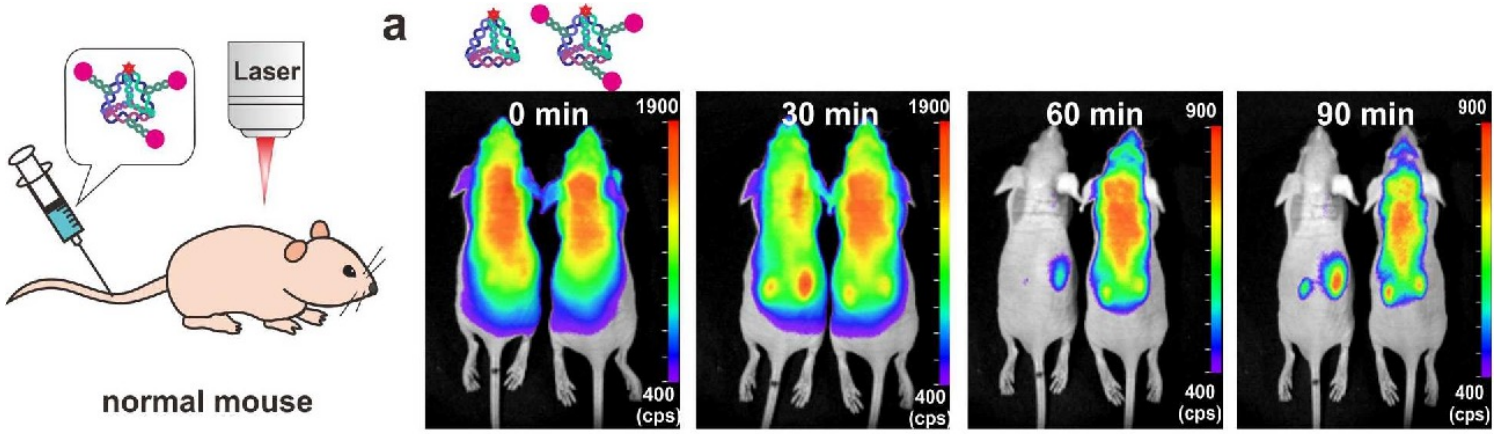


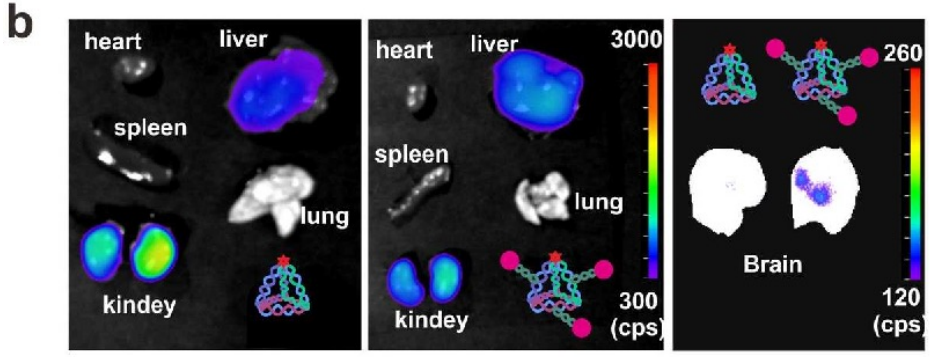


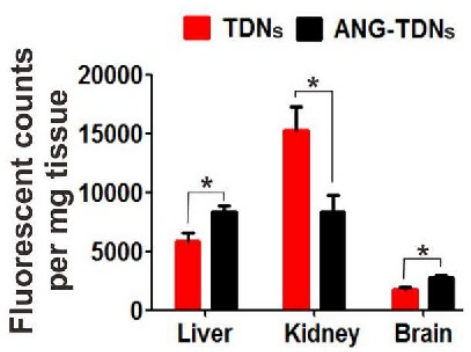
How to measure the targeting efficiency?

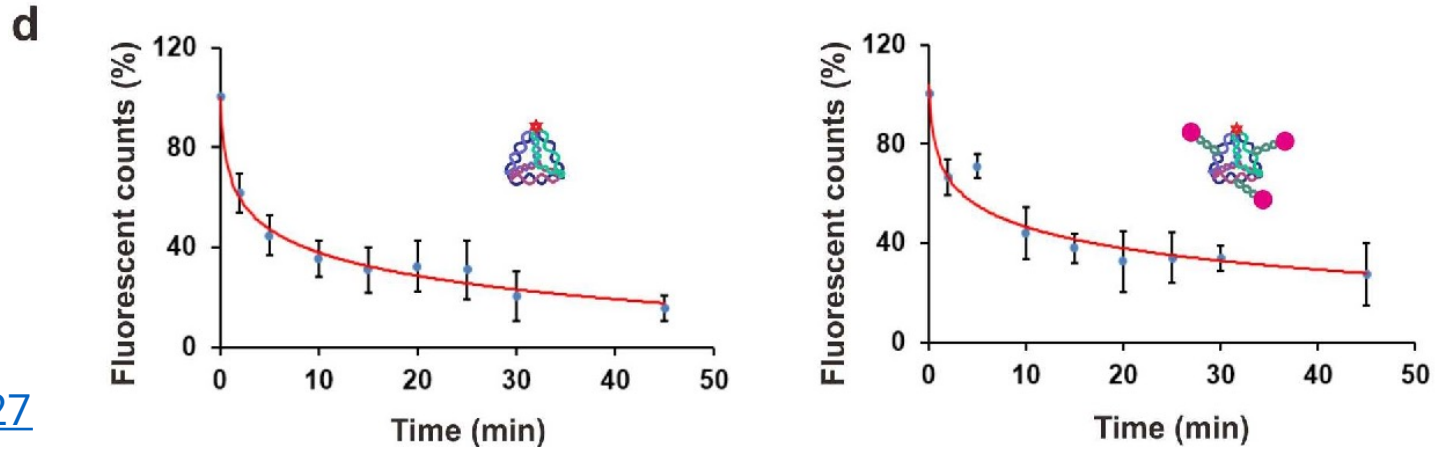
In vivo

IVIS









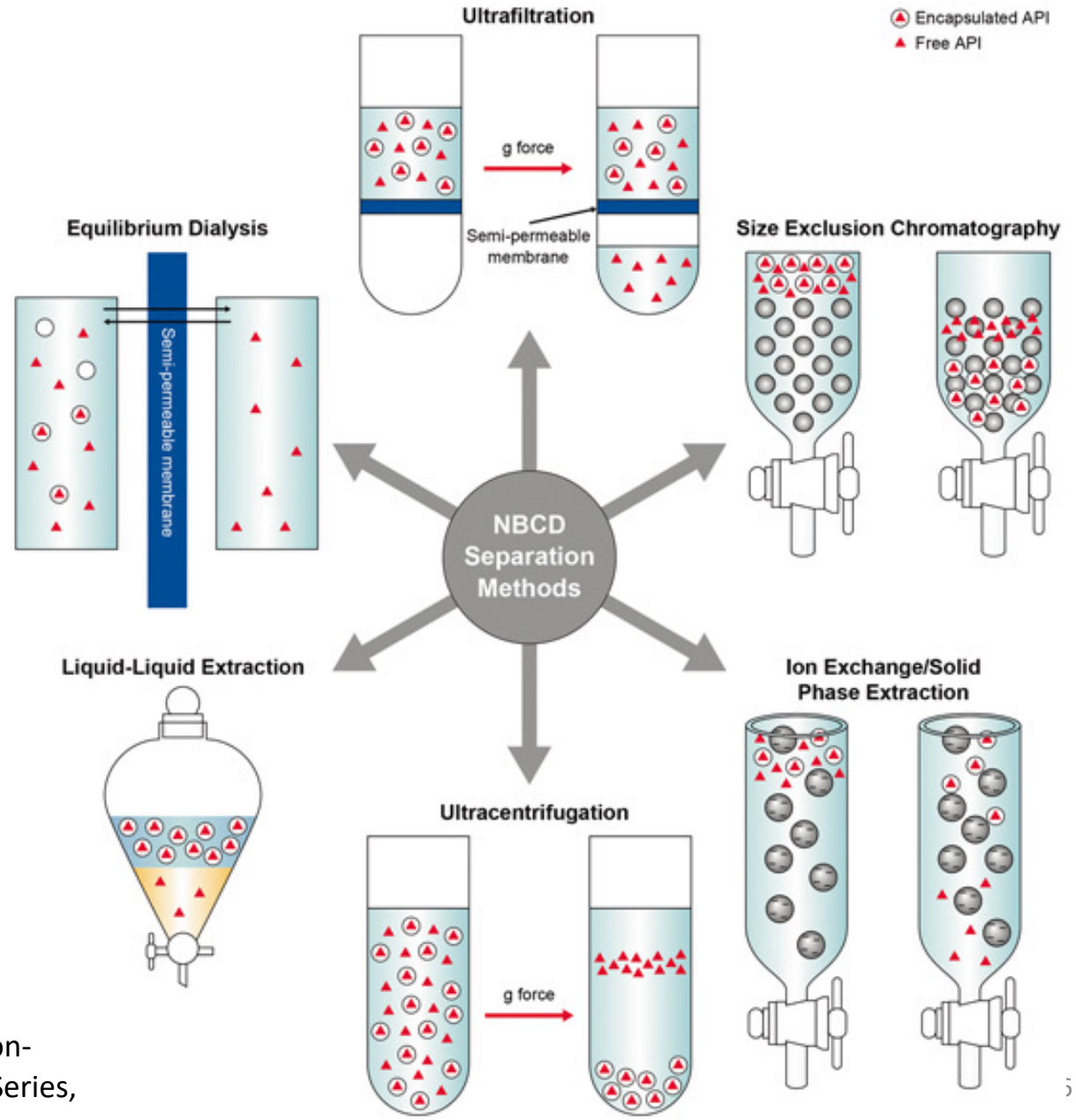
•DOI:10.1021/acsami.7b17927

How to measure drug release?

In Vitro

Detection of the drug separated from carrier

Place sample in liquid environment, take samples at various time points

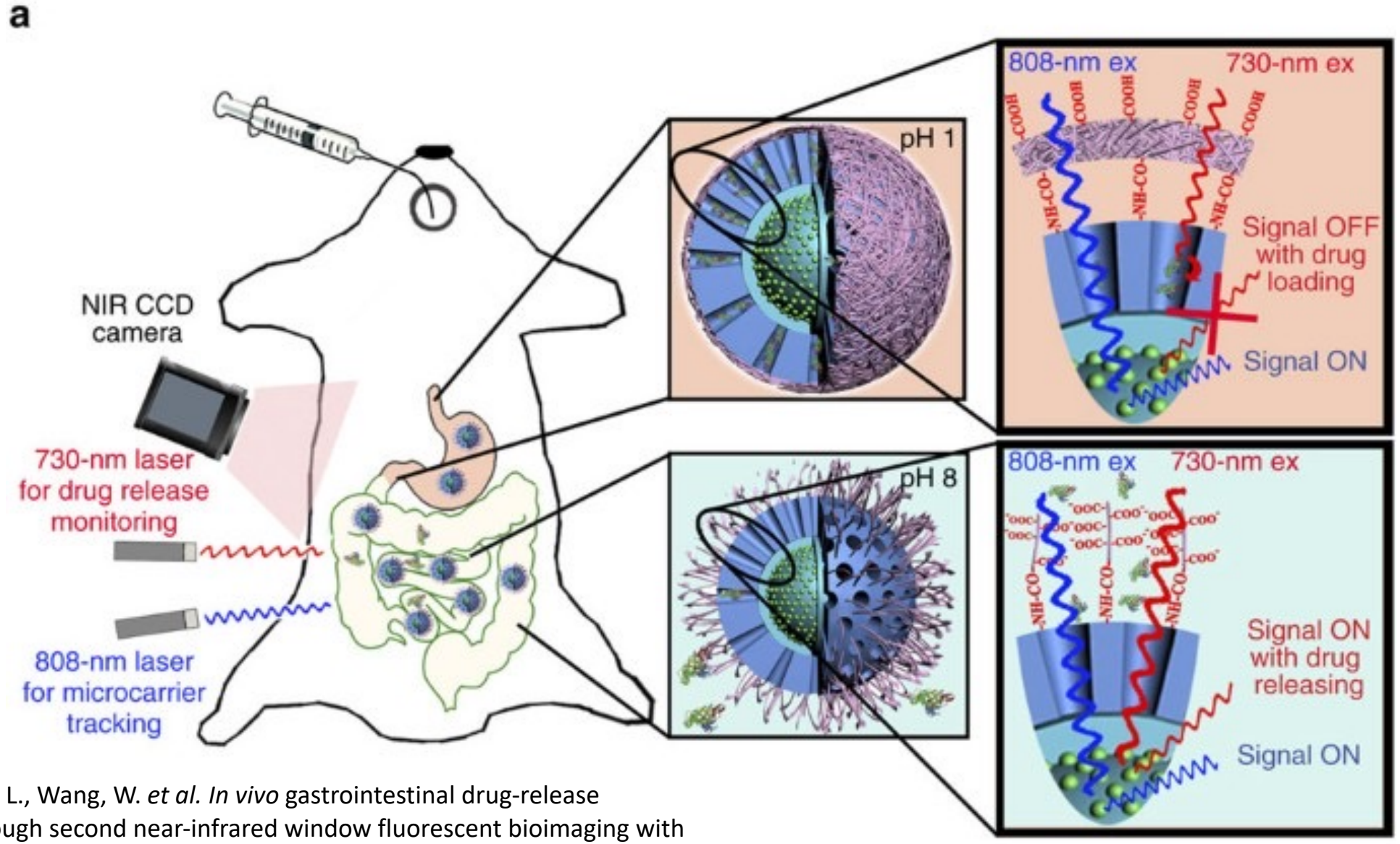


Ambardekar V., Stern S. (2015) NBCD Pharmacokinetics and Bioanalytical Methods to Measure Drug Release. In: Crommelin D., de Vlieger J. (eds) Non-Biological Complex Drugs. AAPS Advances in the Pharmaceutical Sciences Series, vol 20. Springer, Cham. https://doi.org/10.1007/978-3-319-16241-6_8

How to measure drug release *in vivo*?

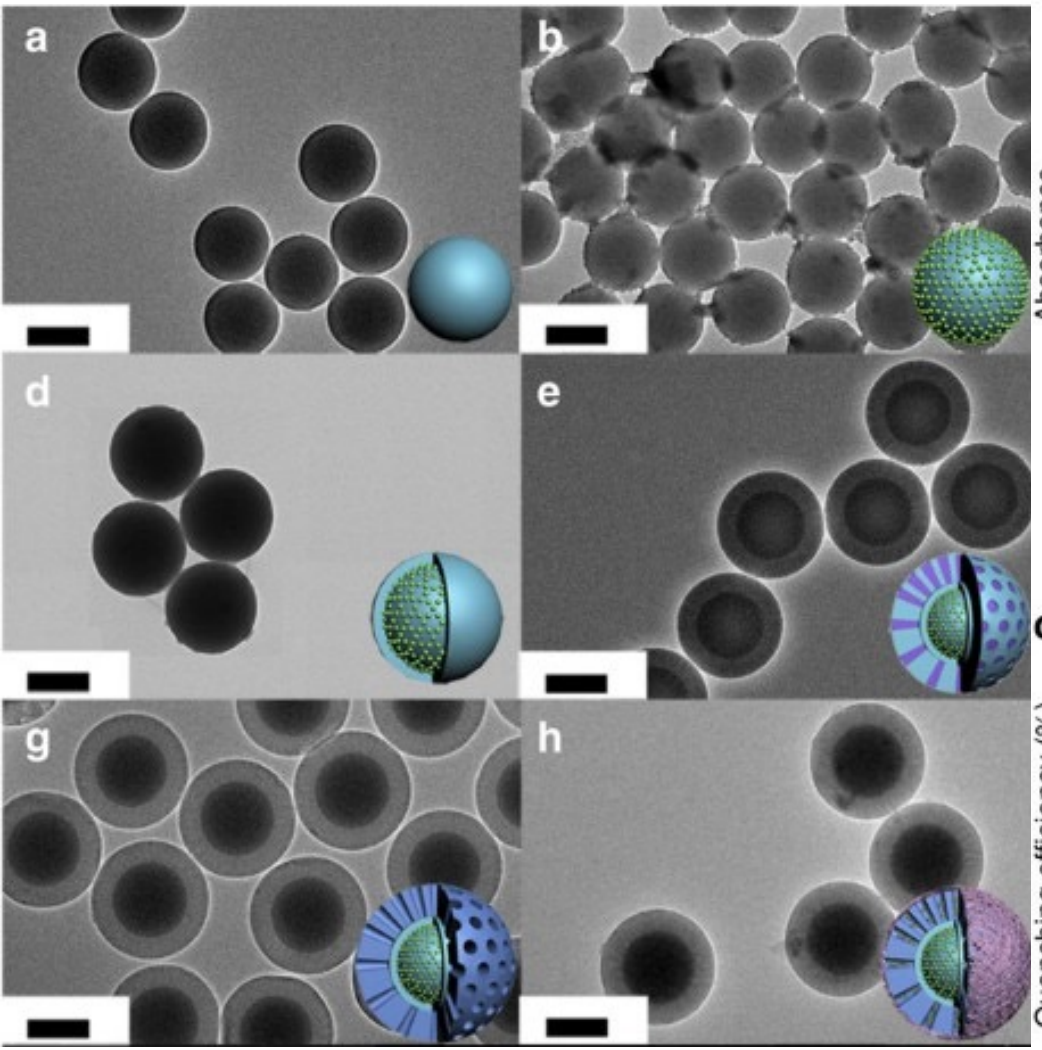
In vivo

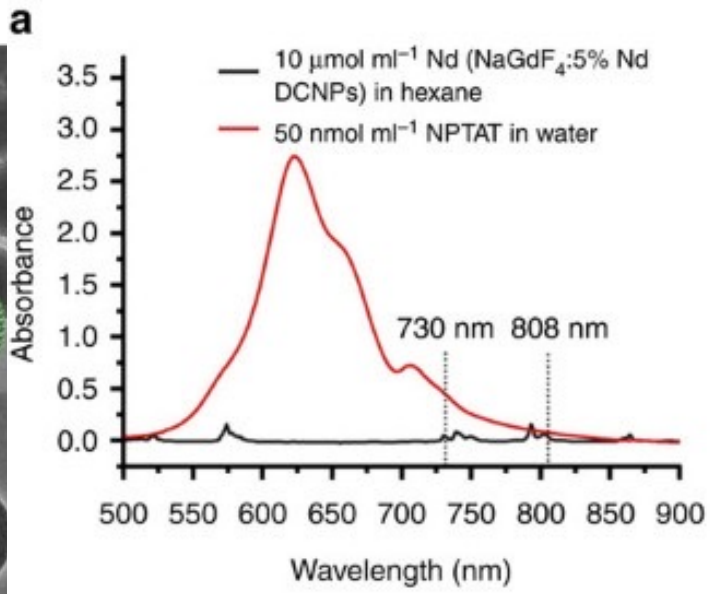
(example) use label/quencher

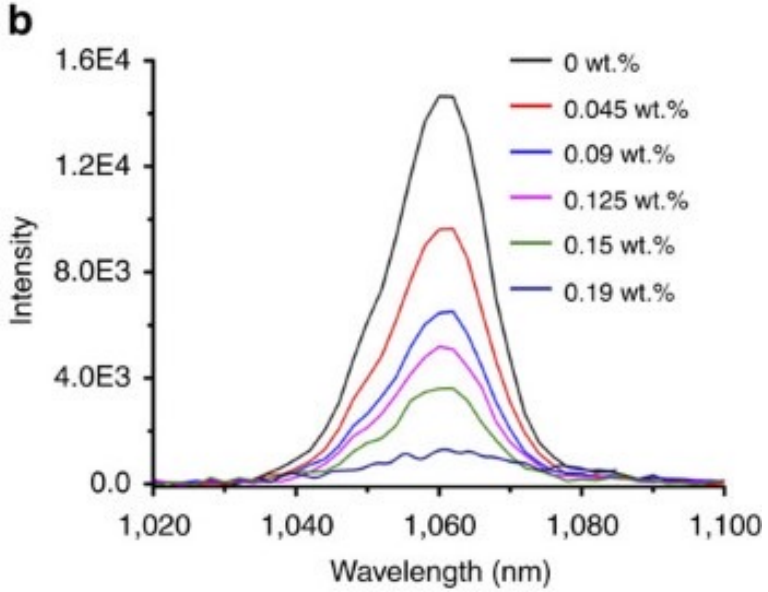


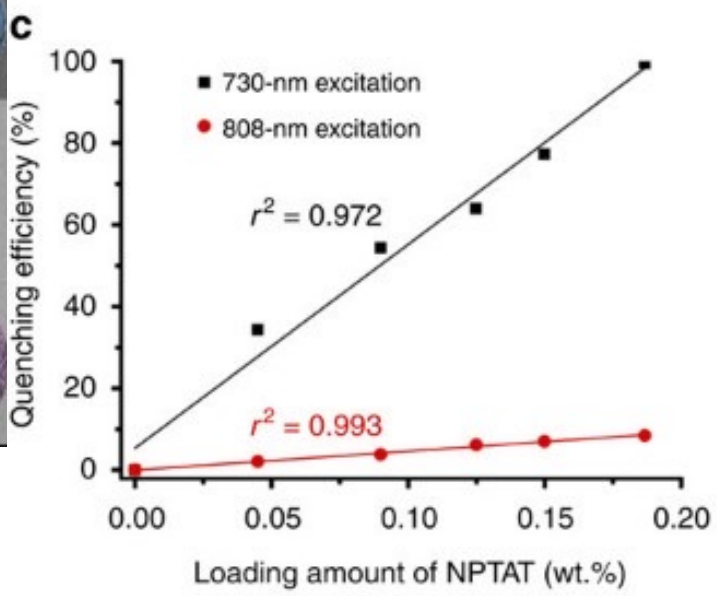
Wang, R., Zhou, L., Wang, W. *et al. In vivo* gastrointestinal drug-release monitoring through second near-infrared window fluorescent bioimaging with orally delivered microcarriers. *Nat Commun* **8,** 14702 (2017).

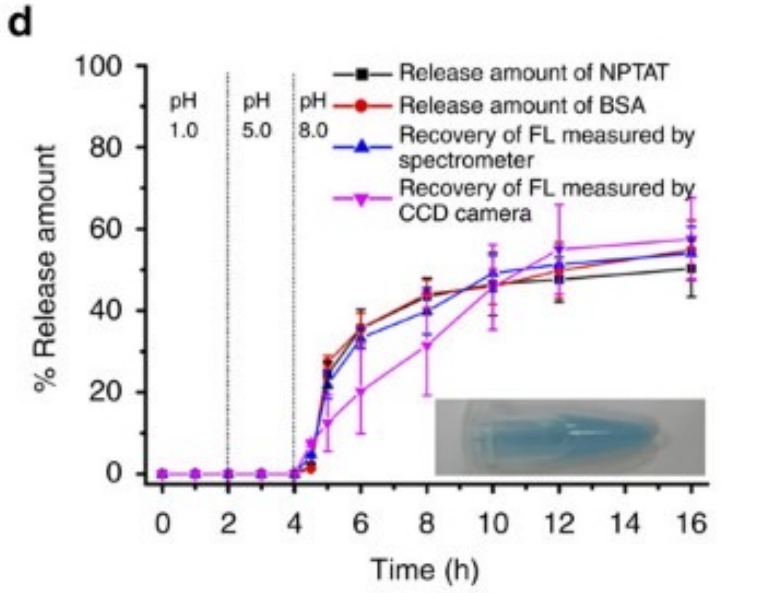
How to measure drug release in vivo?



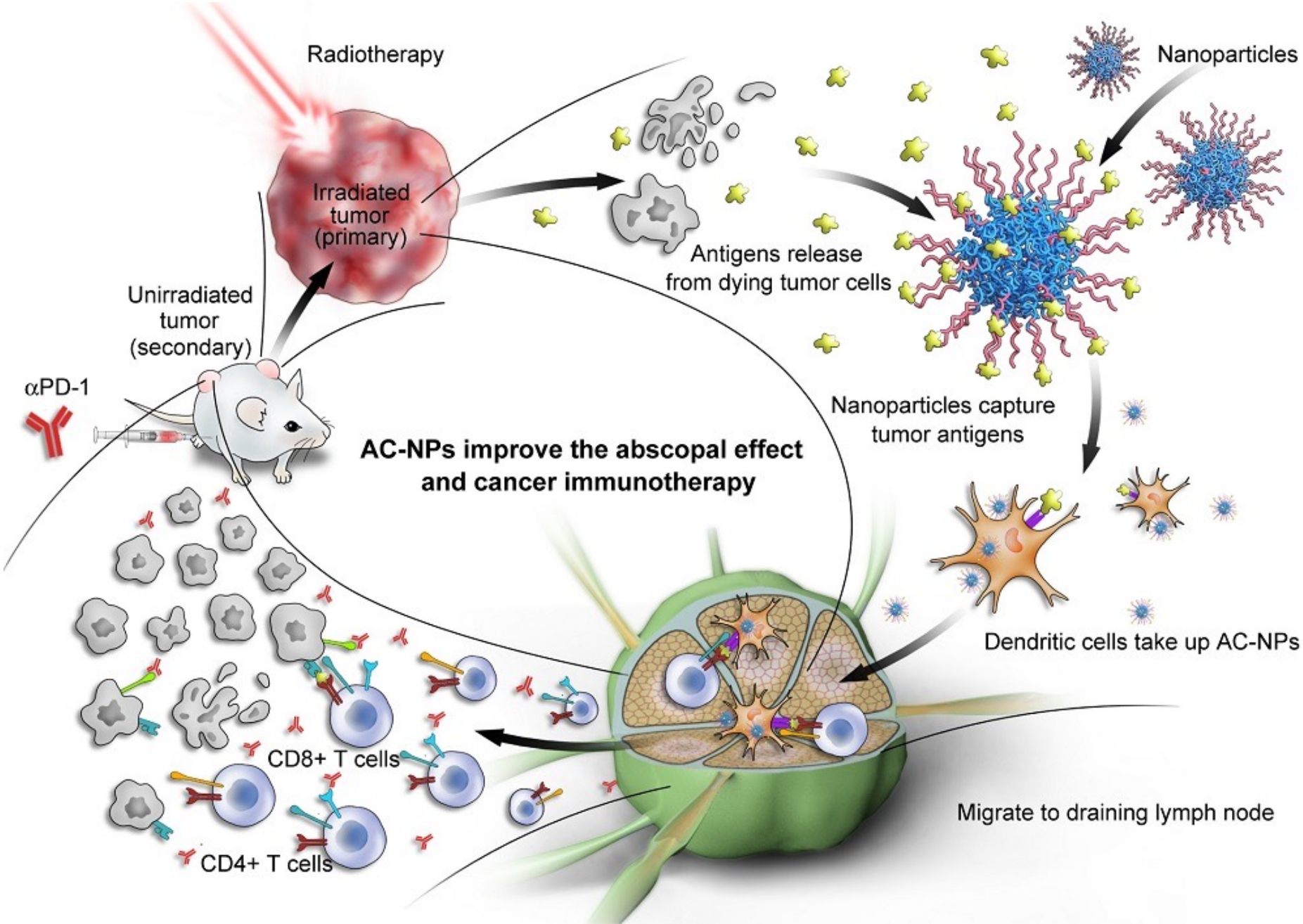








How to characterize an immune response?

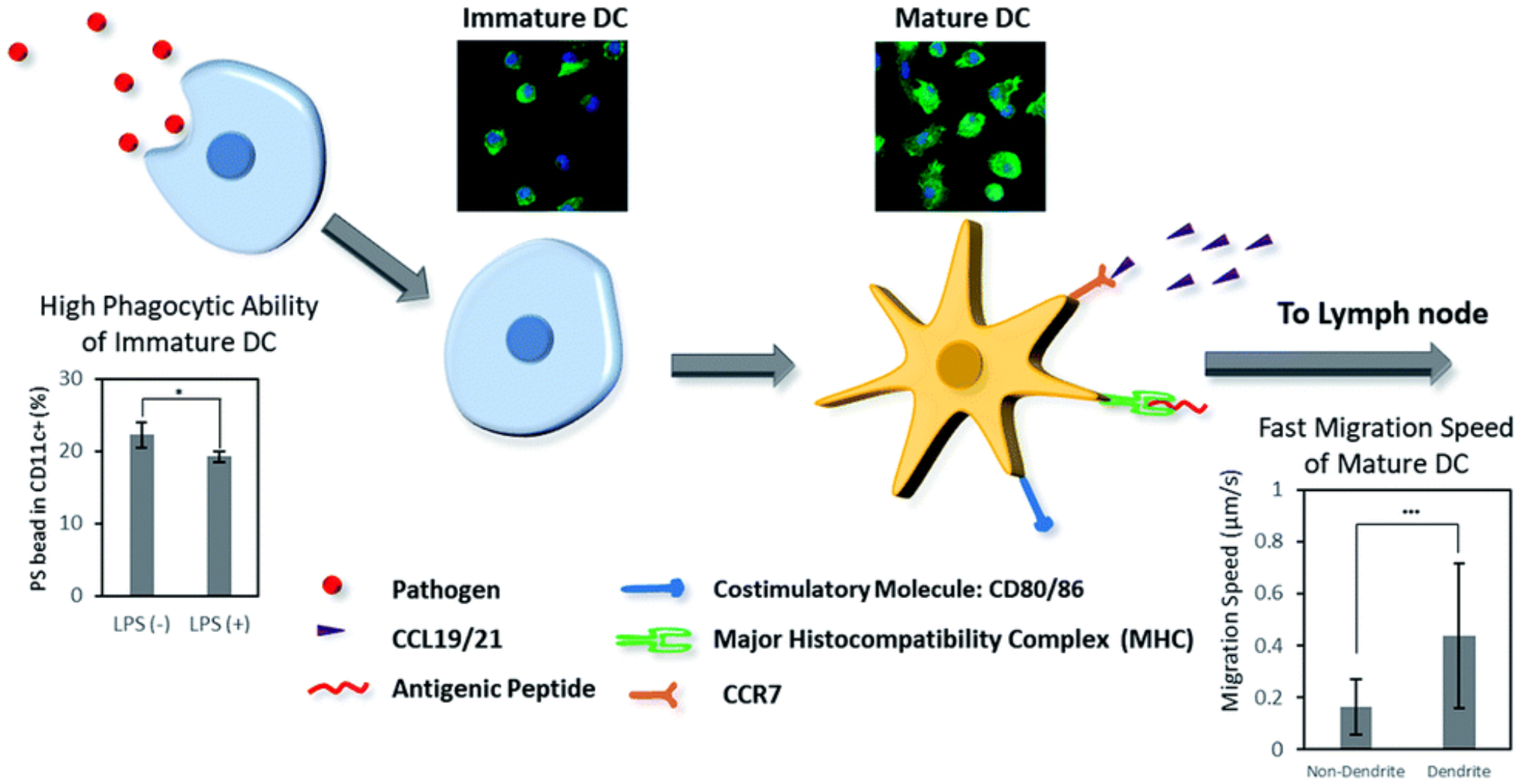


How to characterize an immune response?

Dendritic cell activation

In Vitro

Analysis of dendritic cell maturation signals

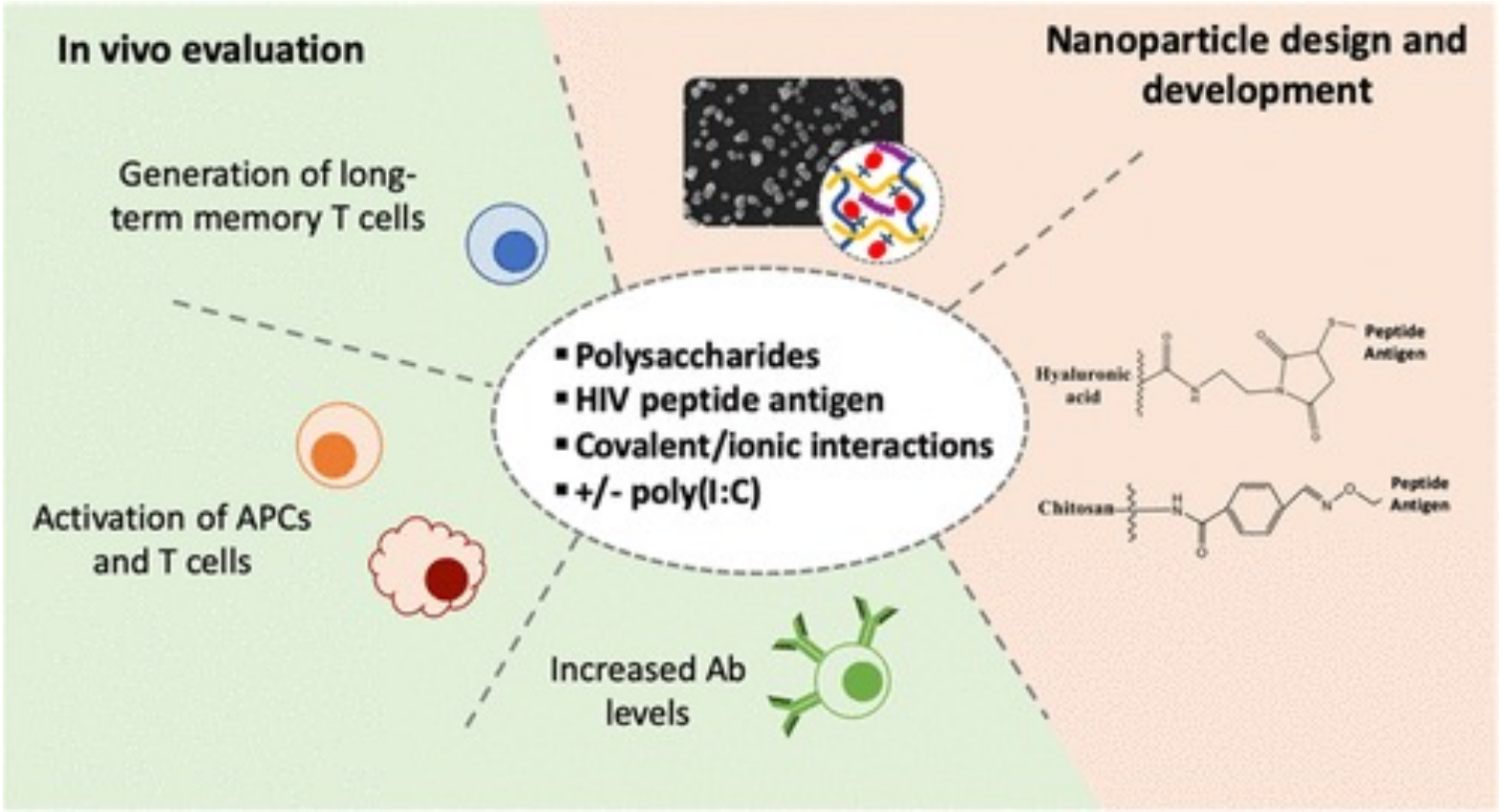


RSC Adv., 2019,**9**, 11230-11238

How to characterize an immune response?

In vivo

Animal experiments



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Conclusion

- Research in biomaterials is **extremely multidisciplinary**, and thus techniques used to characterize materials properties and performance are numerous
- Characterization techniques depend on the intended use: **in vitro or in v**ivo
- Often **multiple techniques** are use to confirm the intended design and function
- Techniques presented today are just a **snapshot** of what is possible!