



Materials in medicine

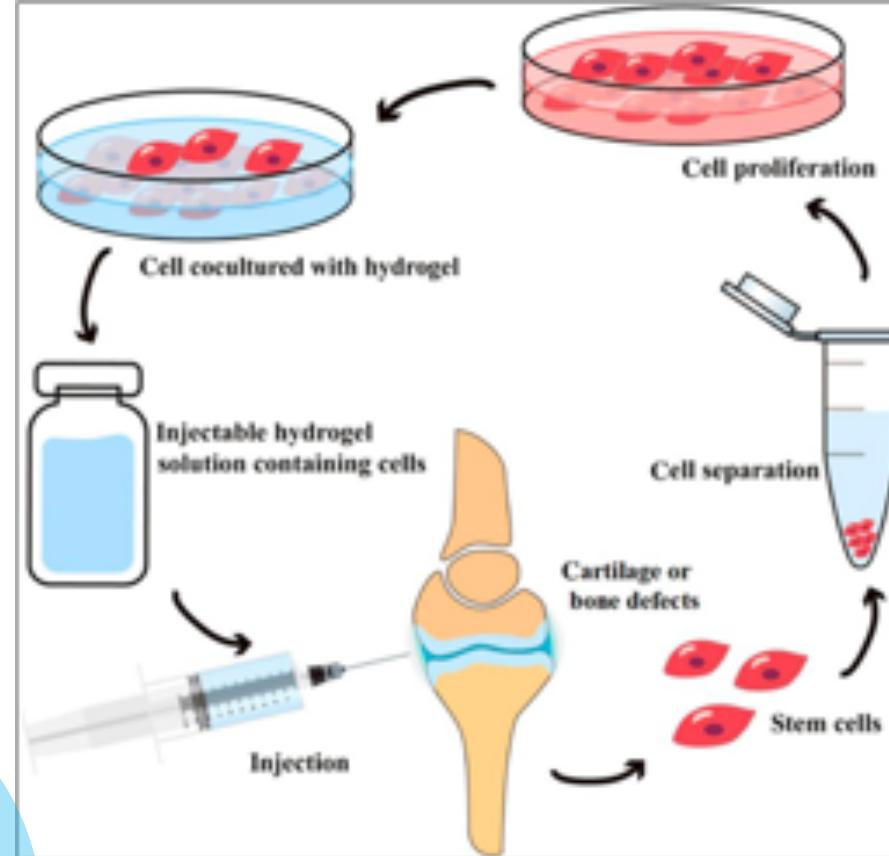
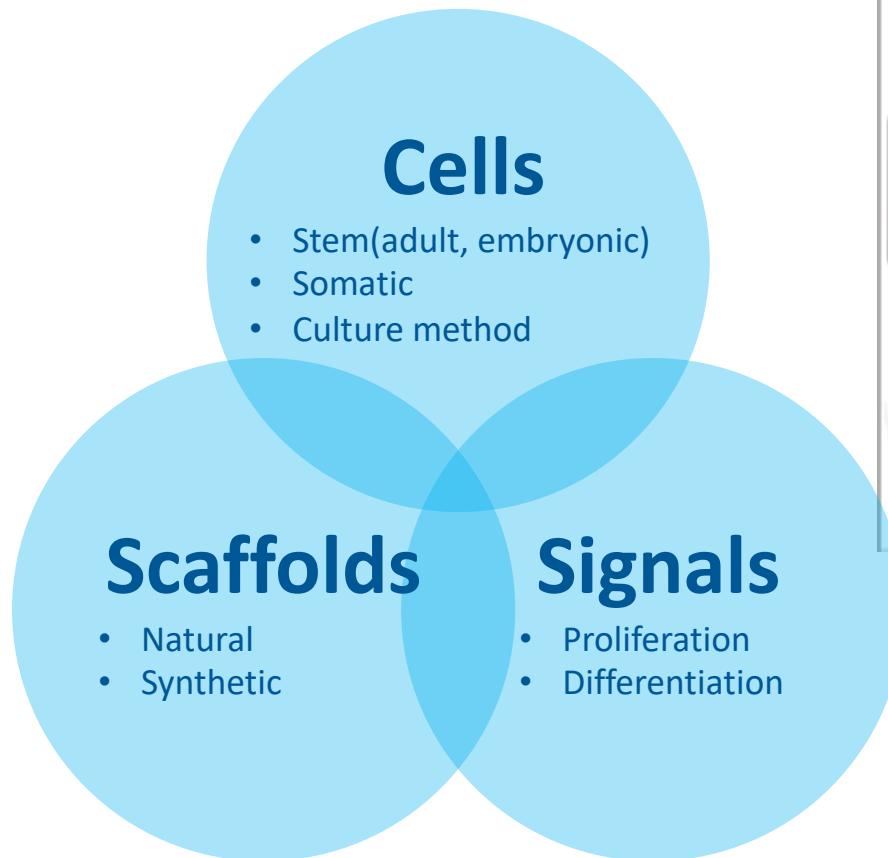
- 1. Biomimetic materials (microscopic characterization)*
- 2. Nanocarriers and Advanced cancer models*

Susana Rocha

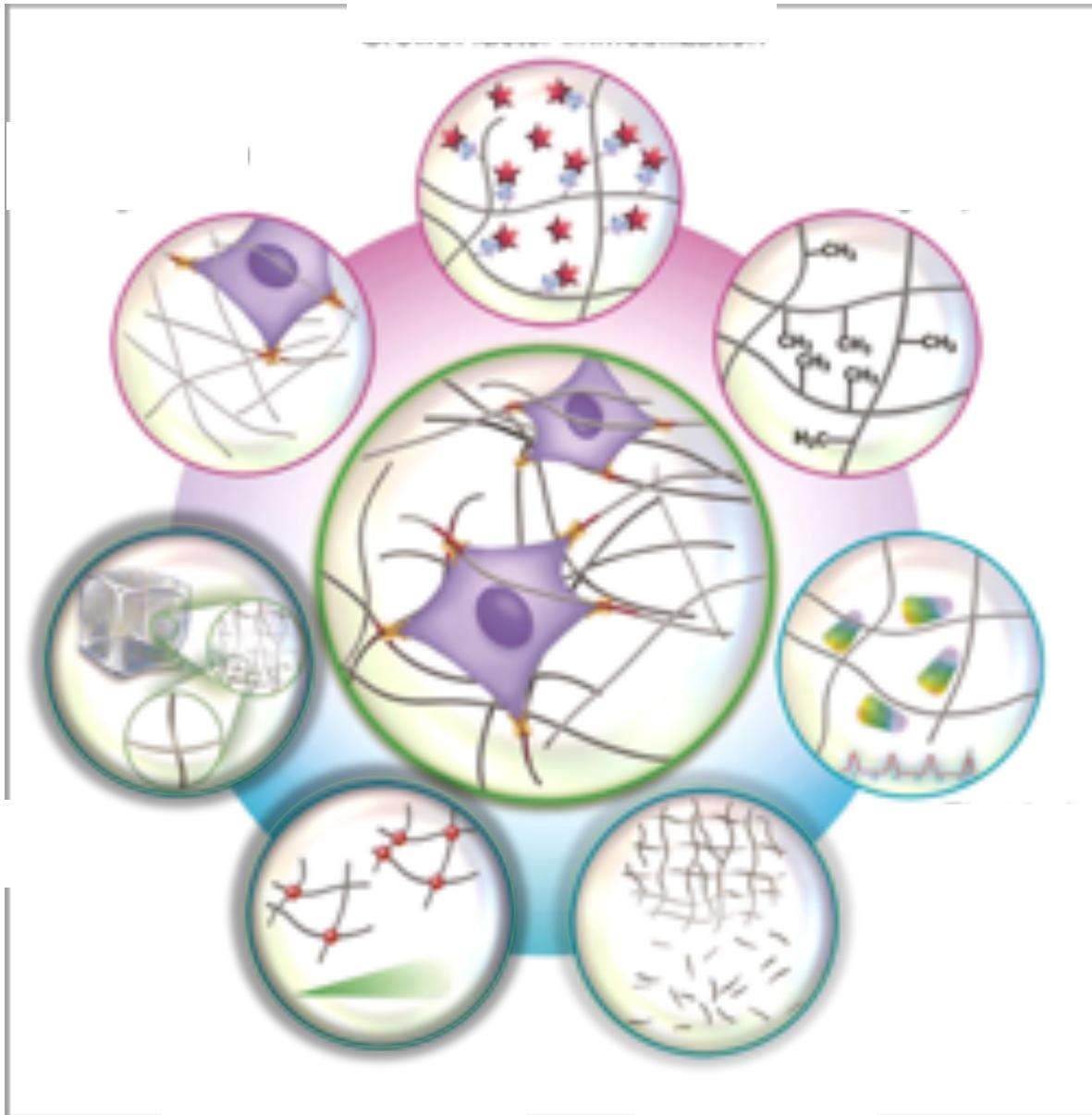
post-doctoral researcher
@ KU Leuven (BE)

Center for Nuclear Sciences and Technologies
18th June 2019

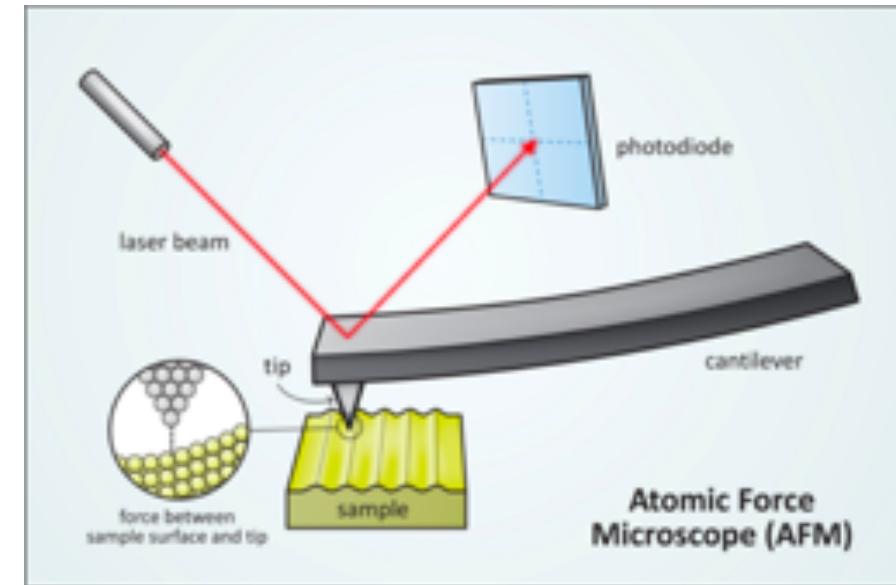
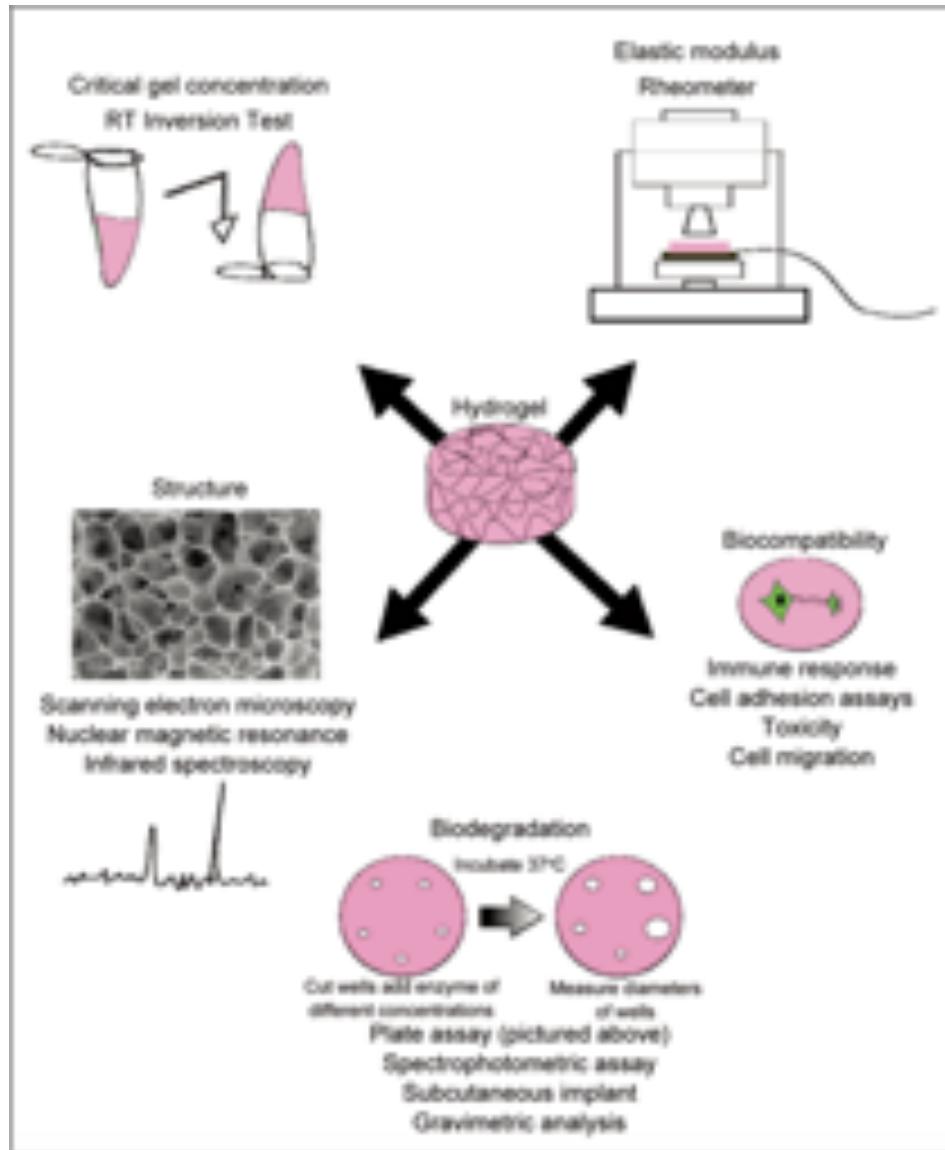
Tissue engineering and 3D cell culture



Design considerations for Biomimetic materials



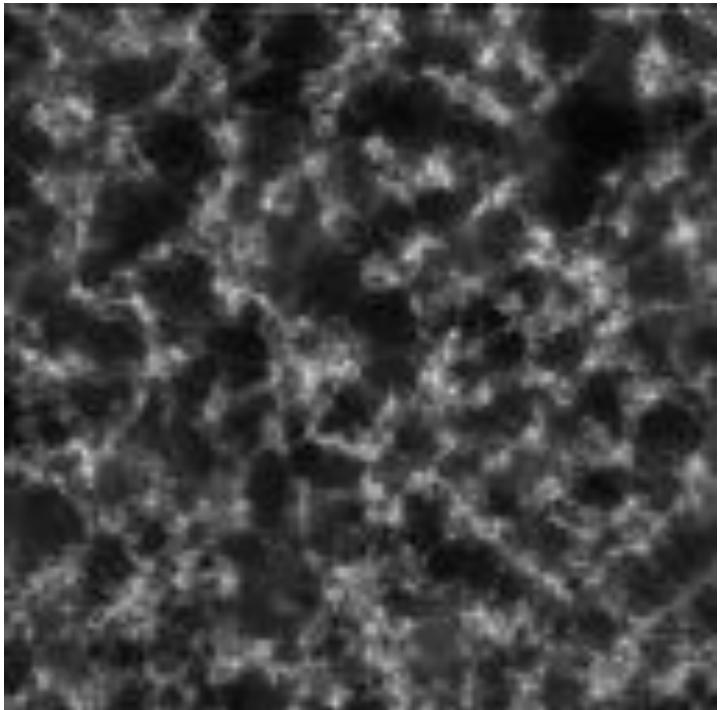
Techniques for characterization of hydrogels



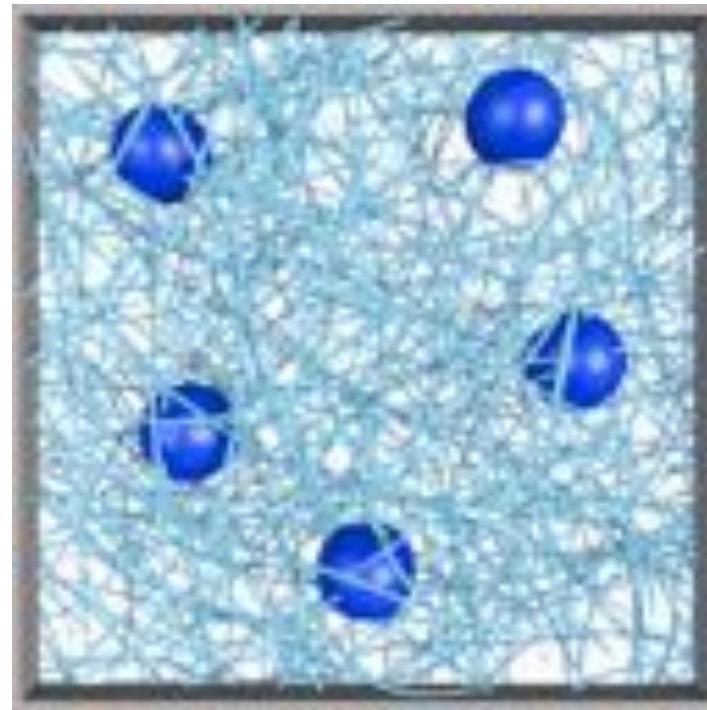
Lack of technologies that can investigate the dynamics of the mechanical and structural properties of biomaterials *in situ*, at the subcellular and molecular level

Material Characterization

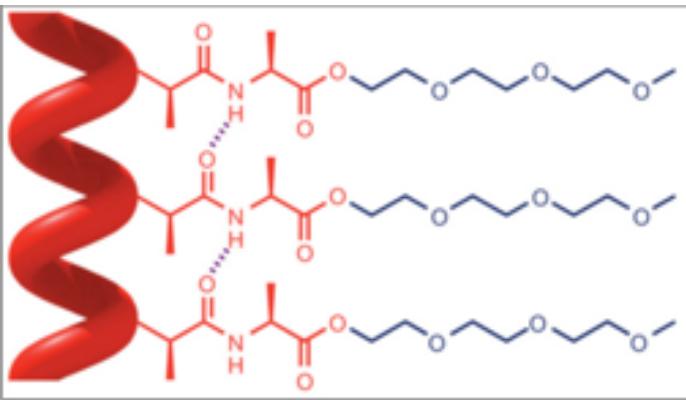
Structure using advanced fluorescence microscopy



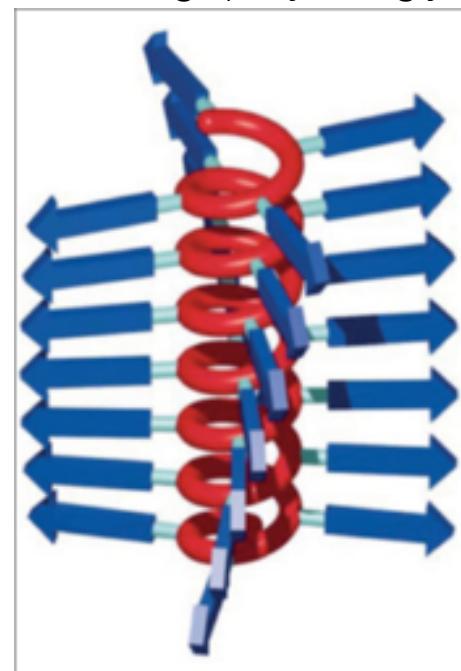
Local mechanical properties using 3D micro-rheology



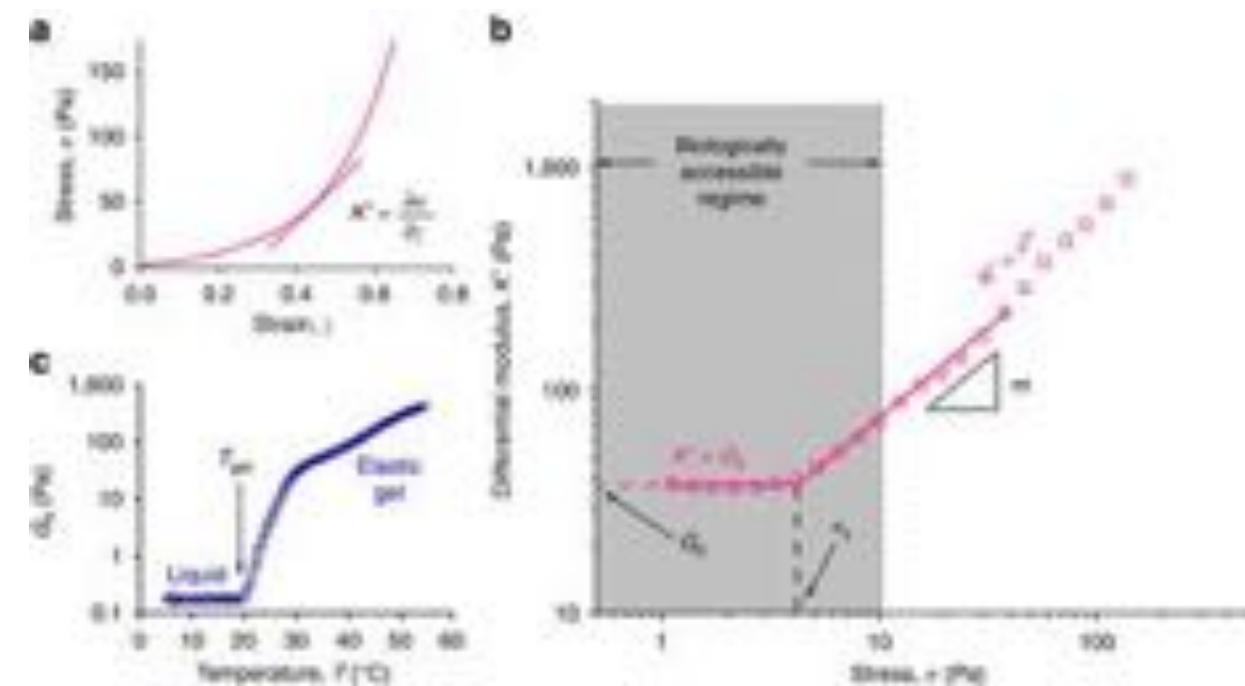
New polymer class for biomimetic 3D matrices



Polyisocyanopeptides (PICs) grafted with oligo(ethylene glycol)

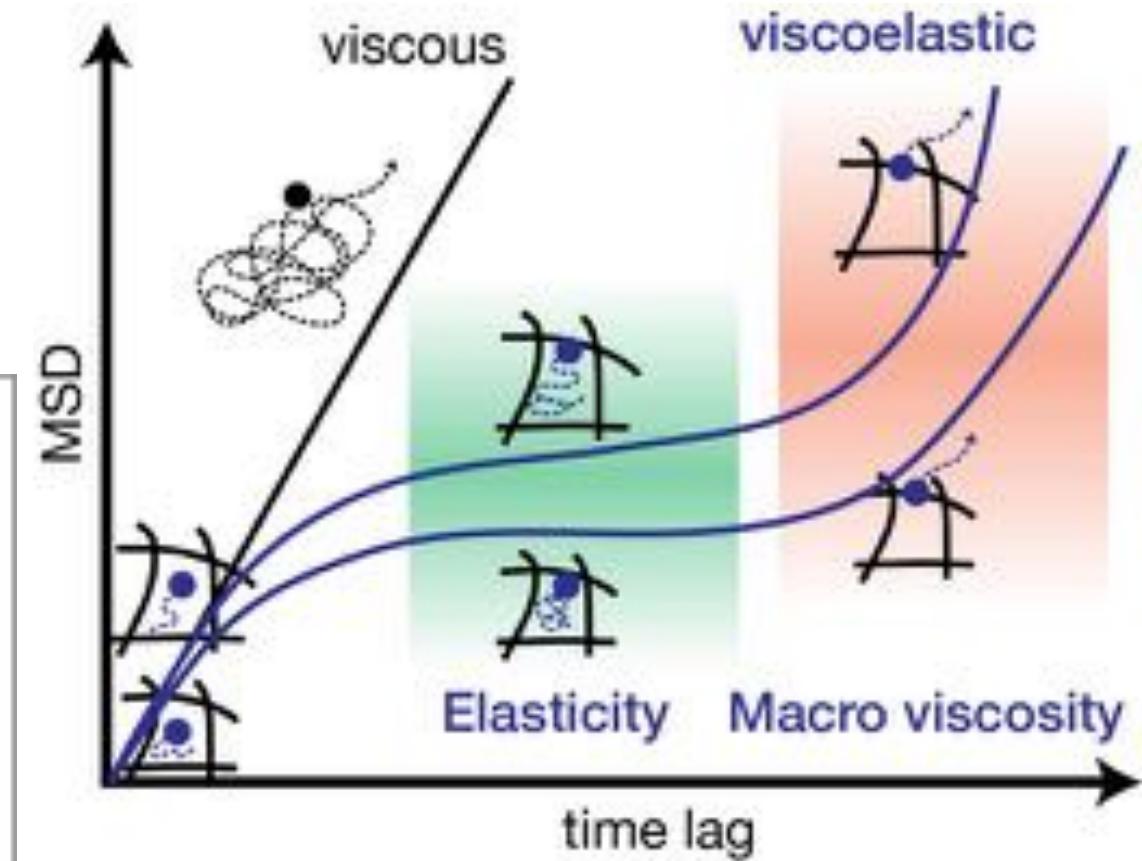
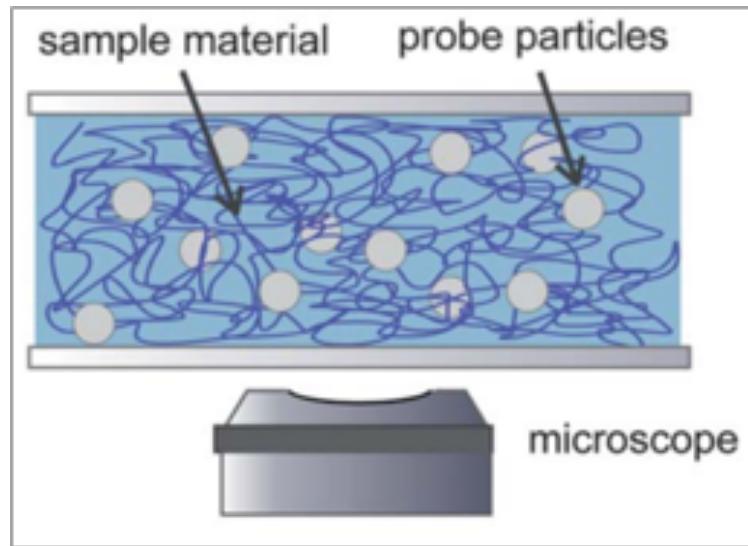
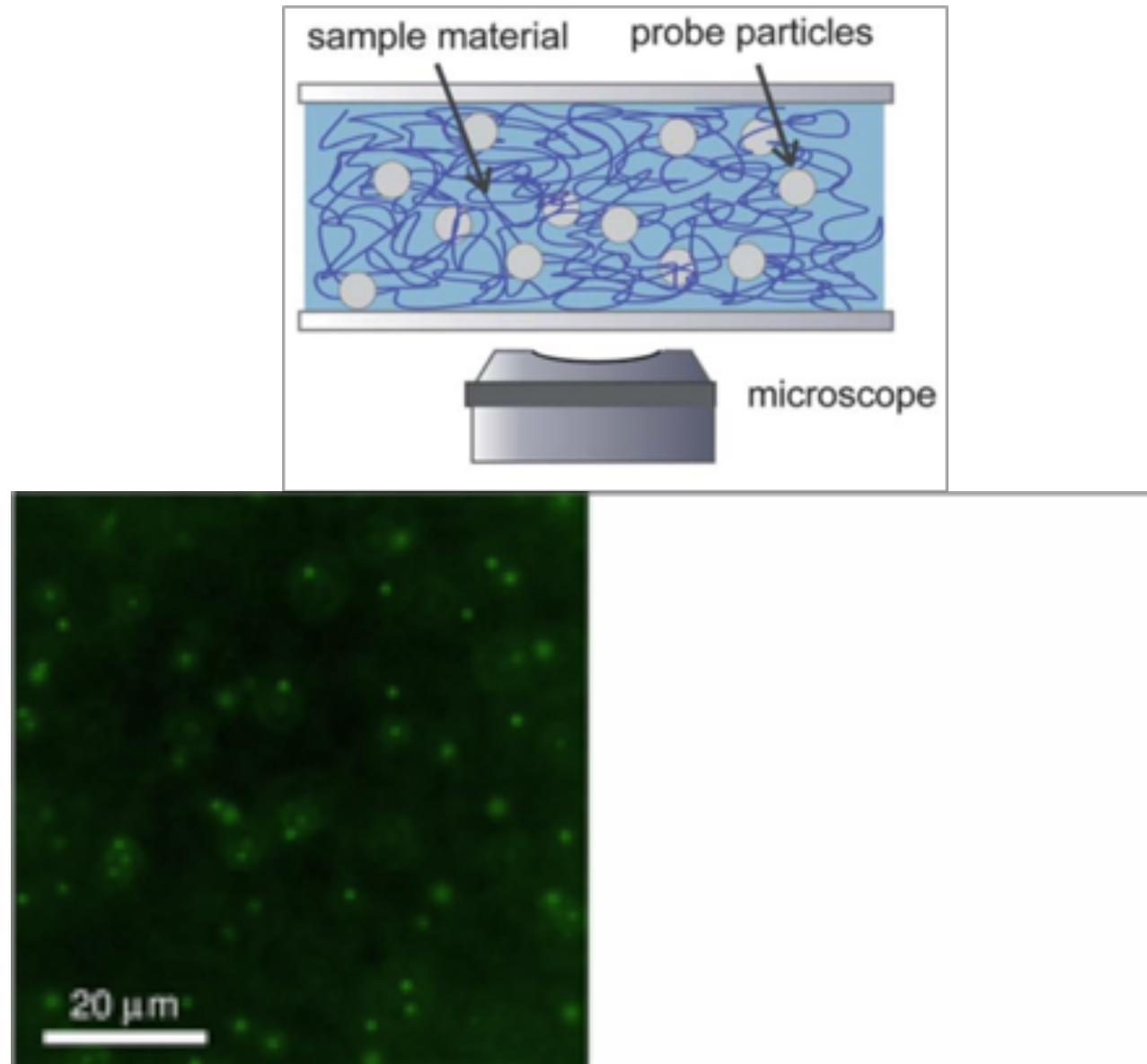


Collaboration with prof. Paul Kouwer,
Radboud University (Nijmegen, The Netherlands)

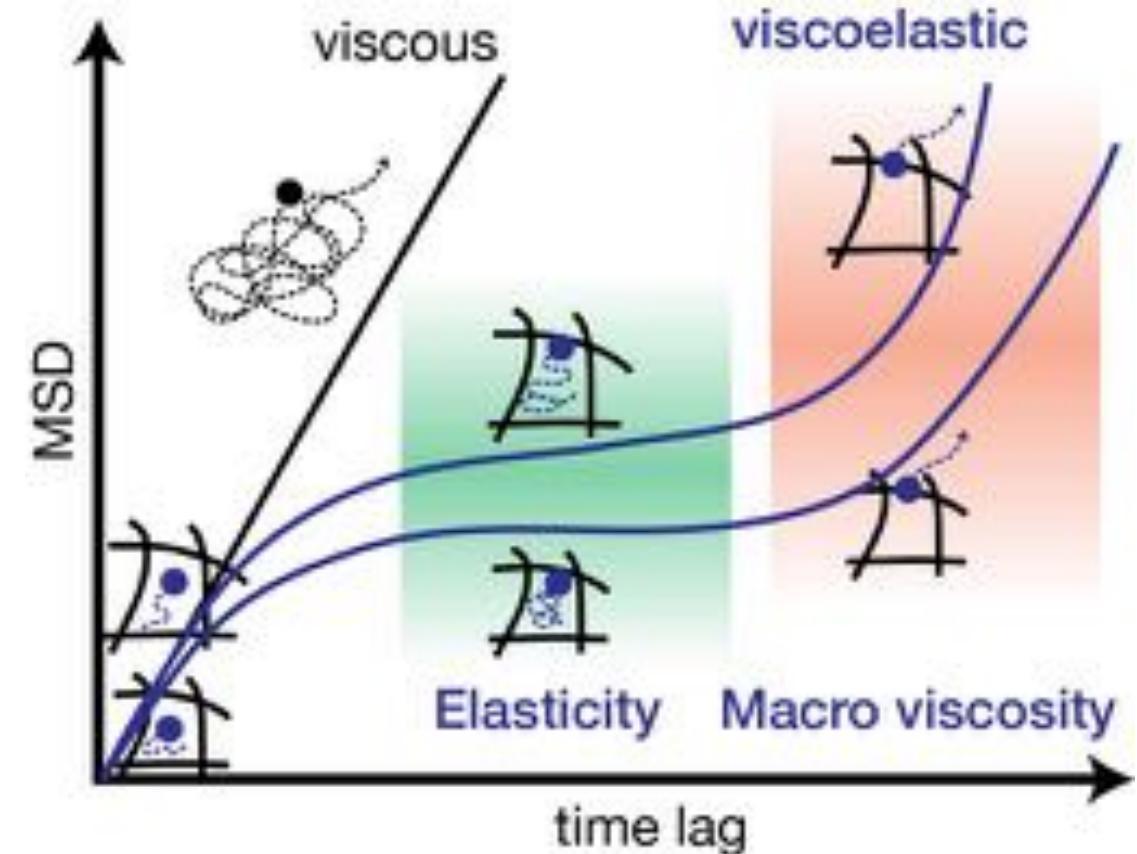
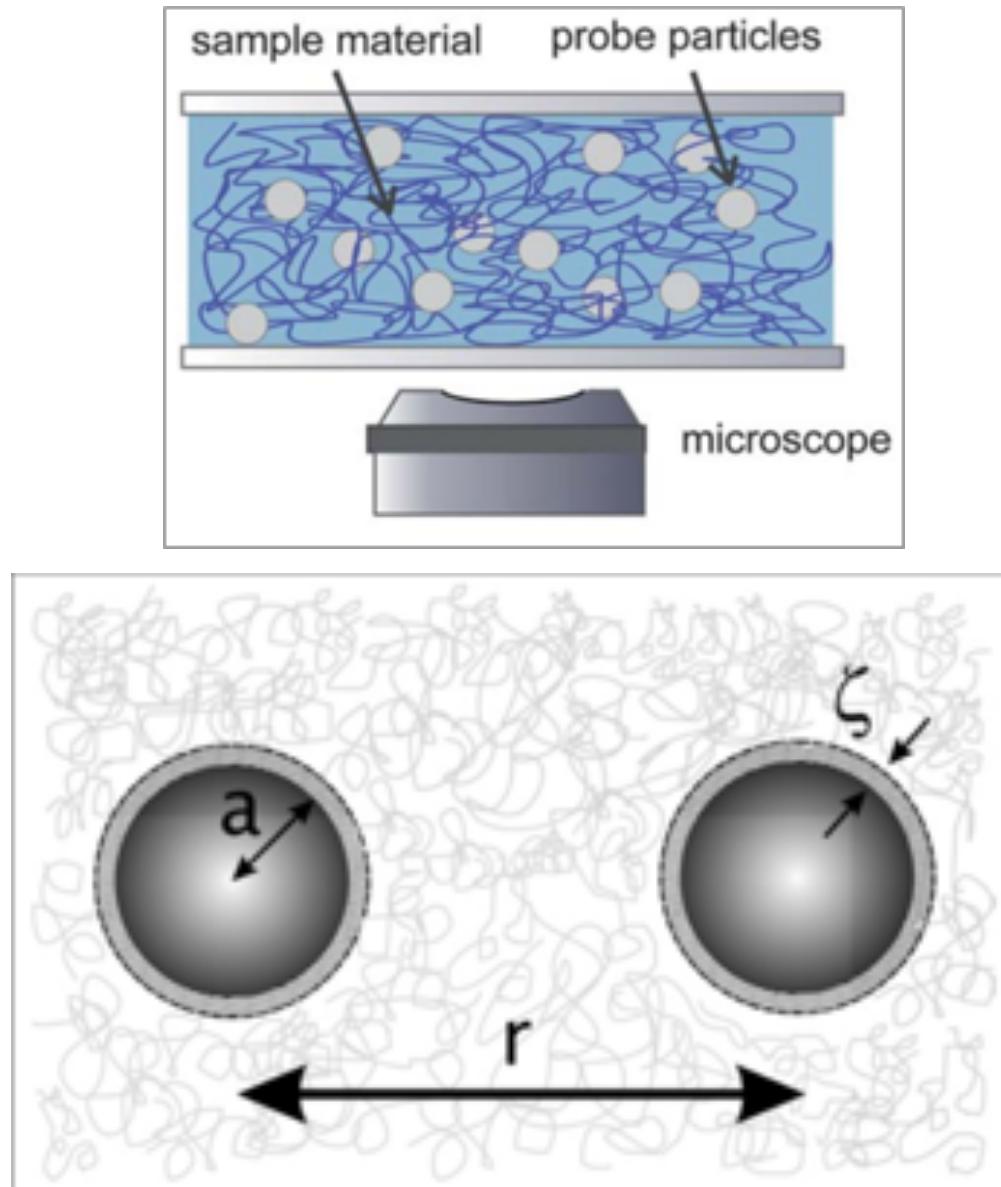


Nature, 493, 651-655 (2014), doi: 10.1038/nature11839
Nature Communications, 5, 5808 (2014), doi: 10.1038/ncomms6808

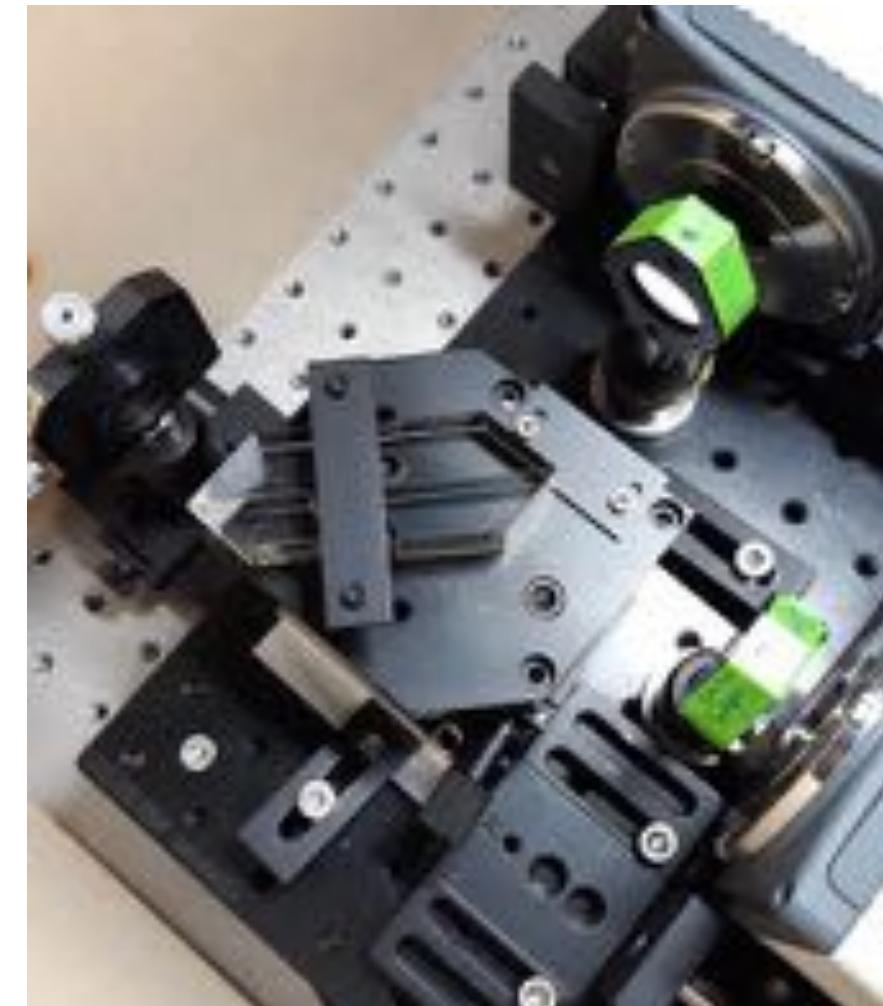
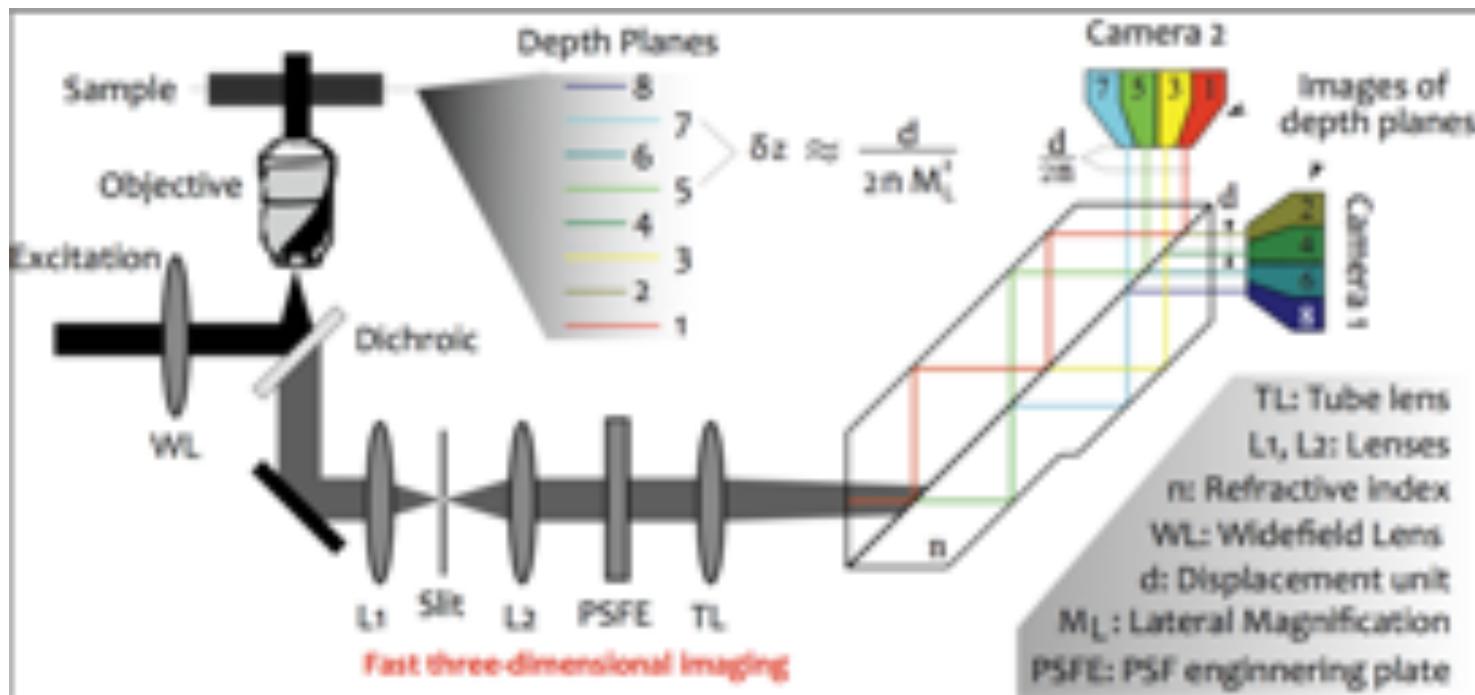
Mechanical Characterization



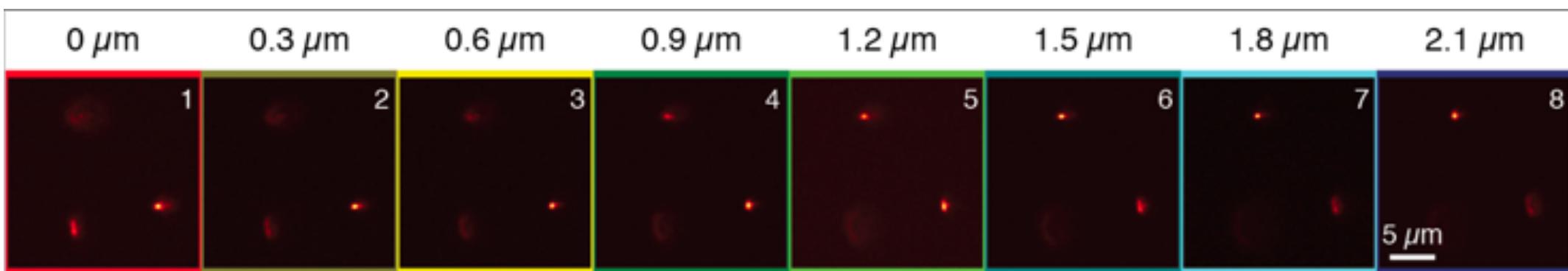
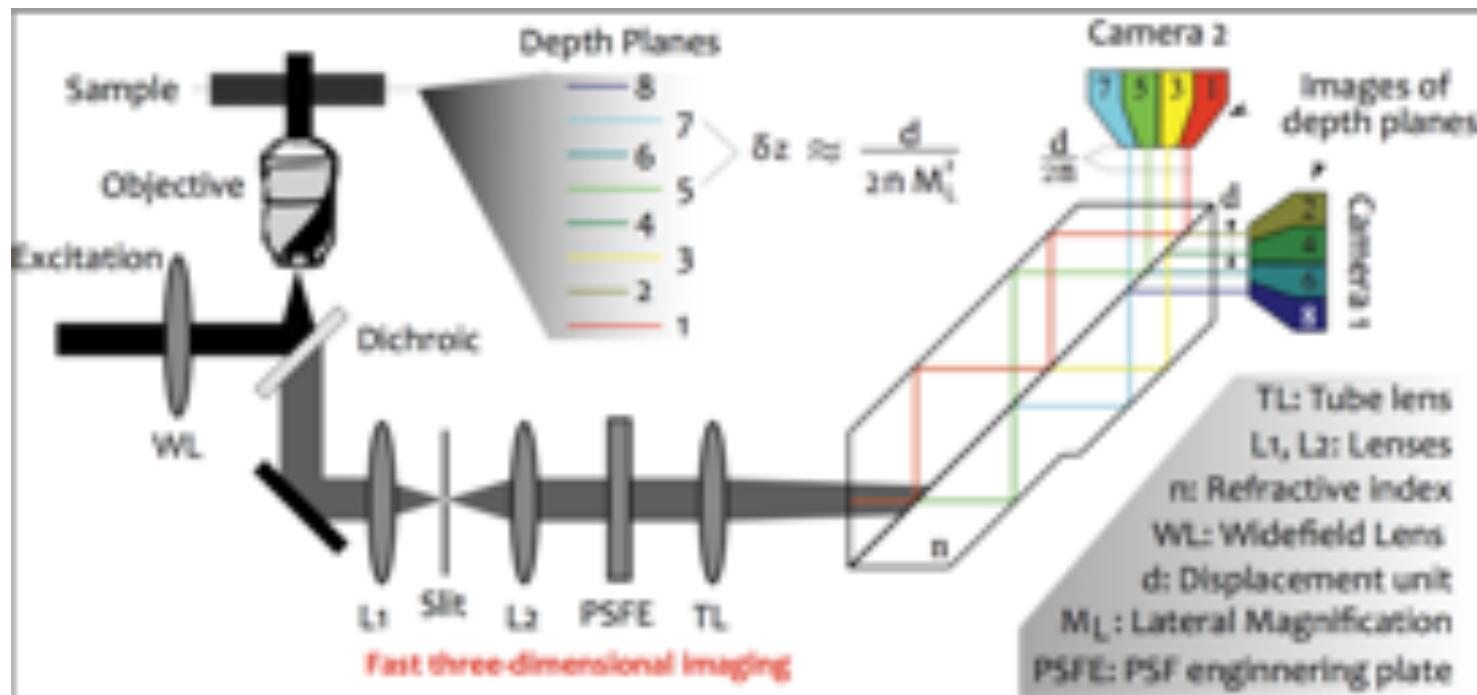
Mechanical Characterization @ the microscale



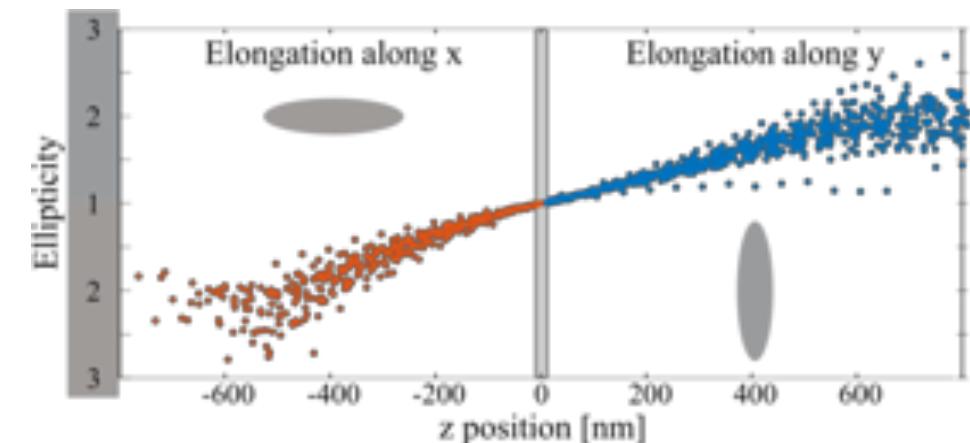
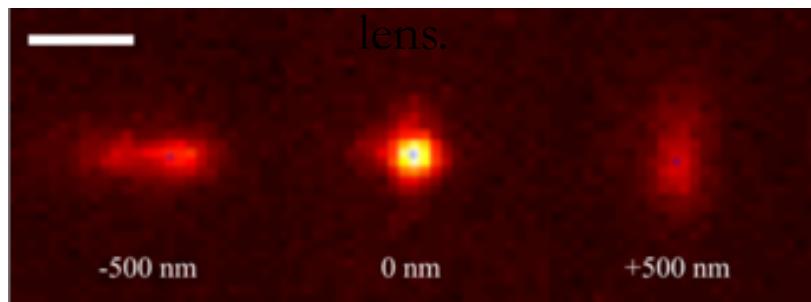
Multi-plane wide field microscope for 2-point micro-rheology



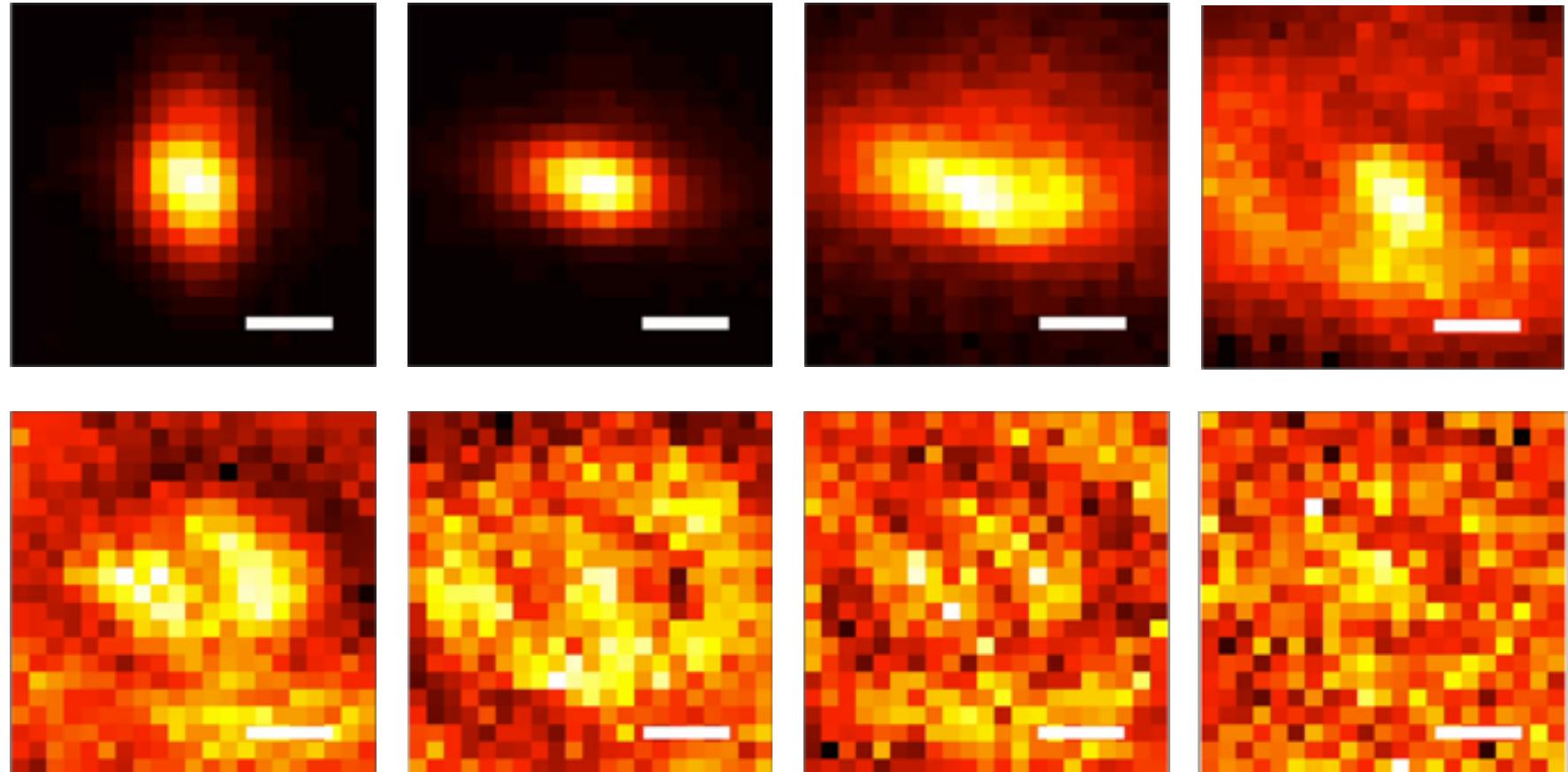
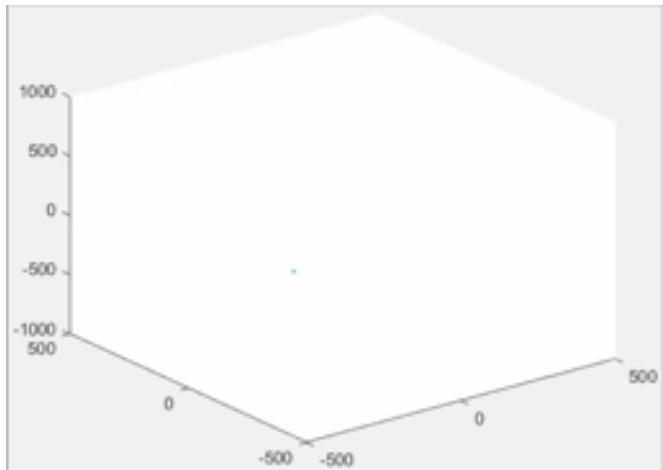
Multi-plane wide field microscope for 2-point micro-rheology



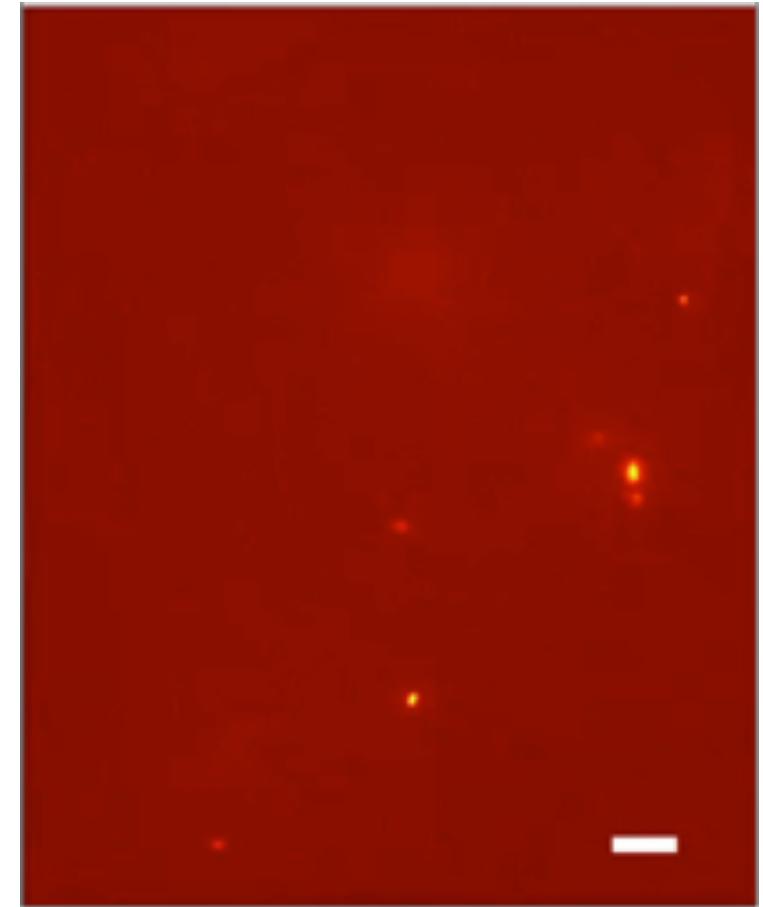
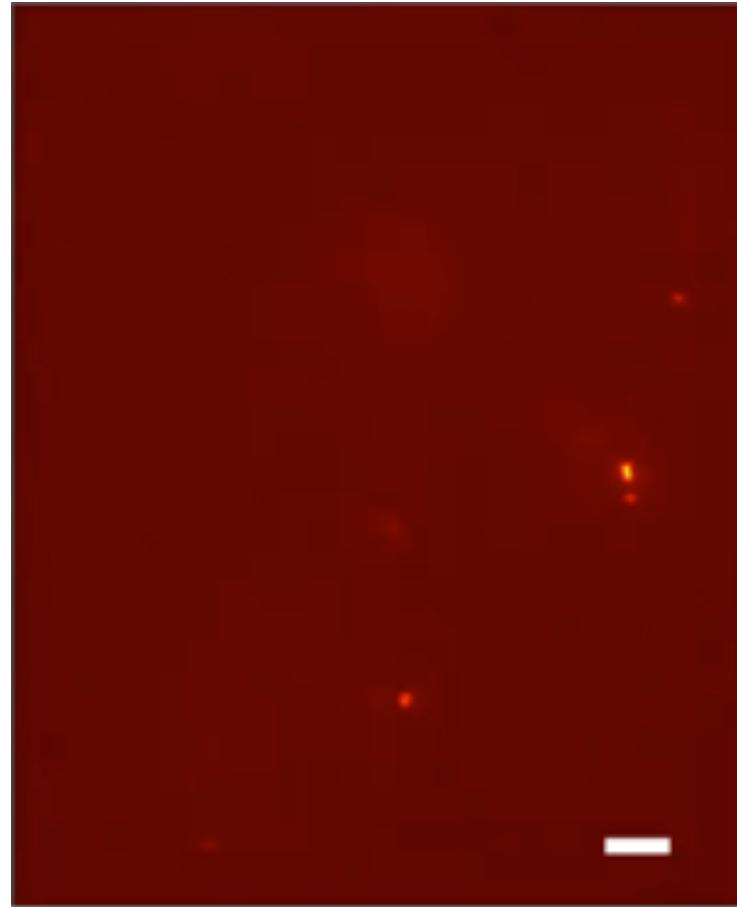
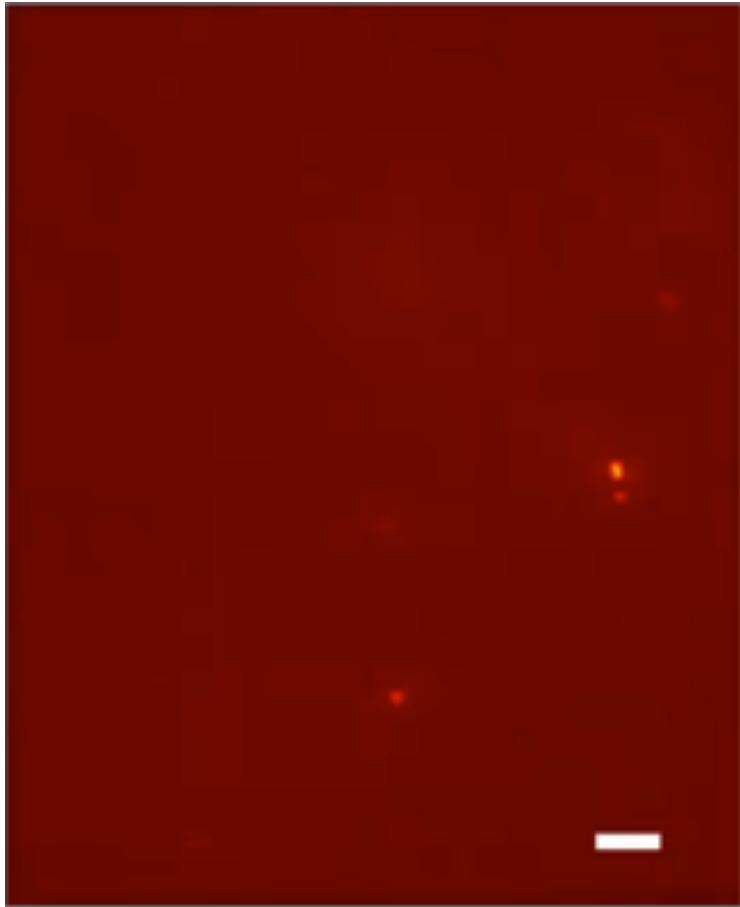
PSF engineering via cylindrical lens.



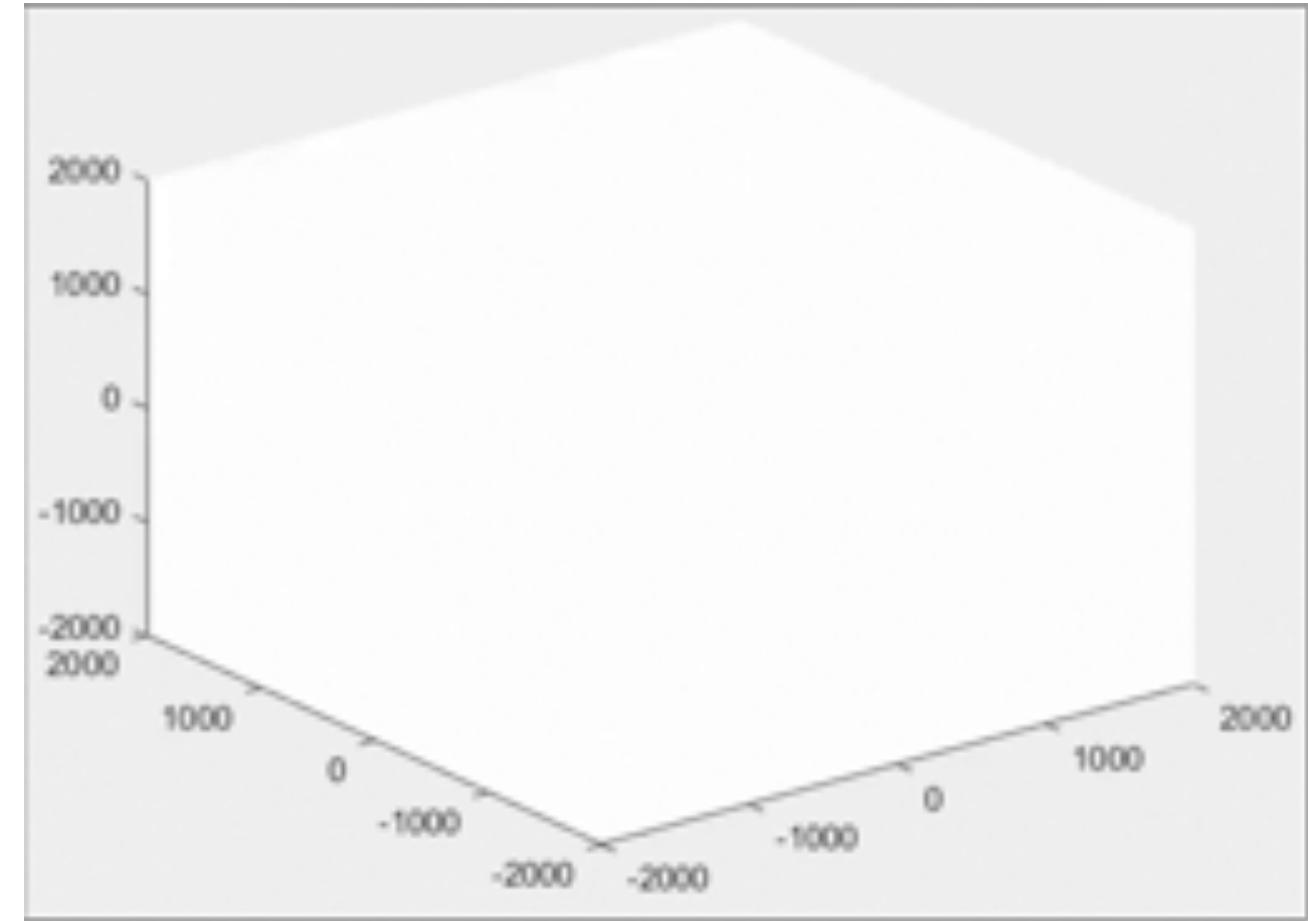
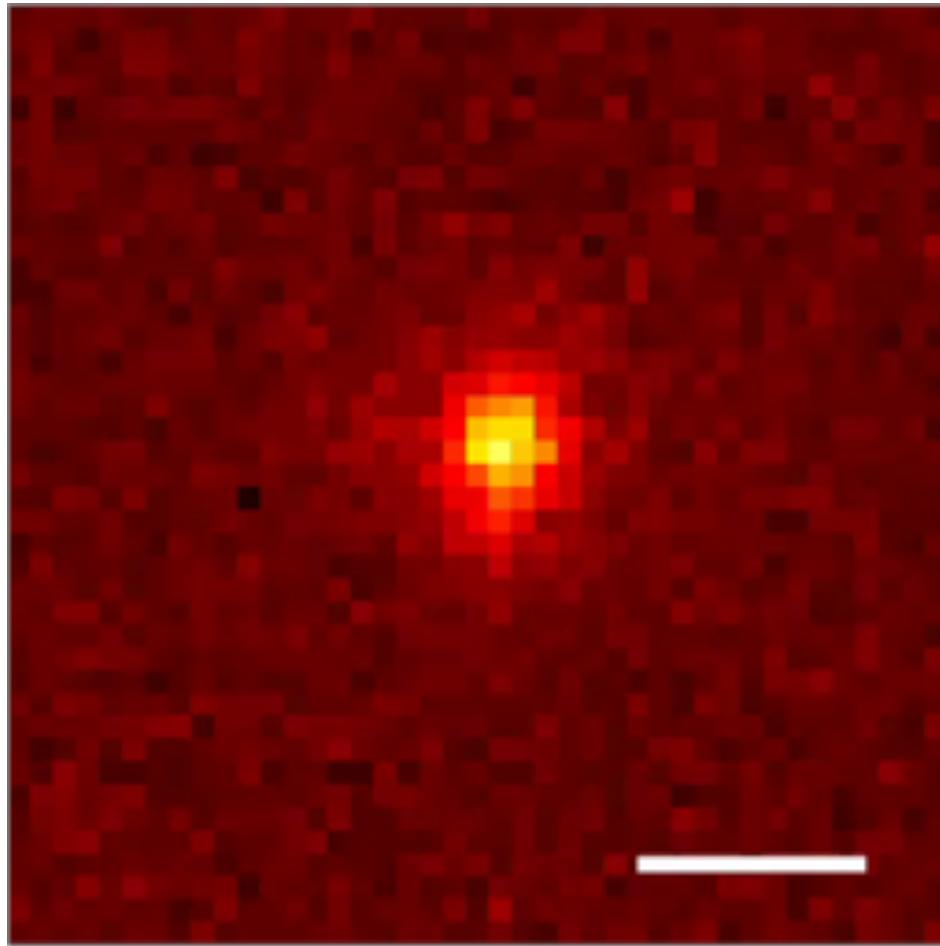
Multi-plane wide field microscope for 2-point micro-rheology



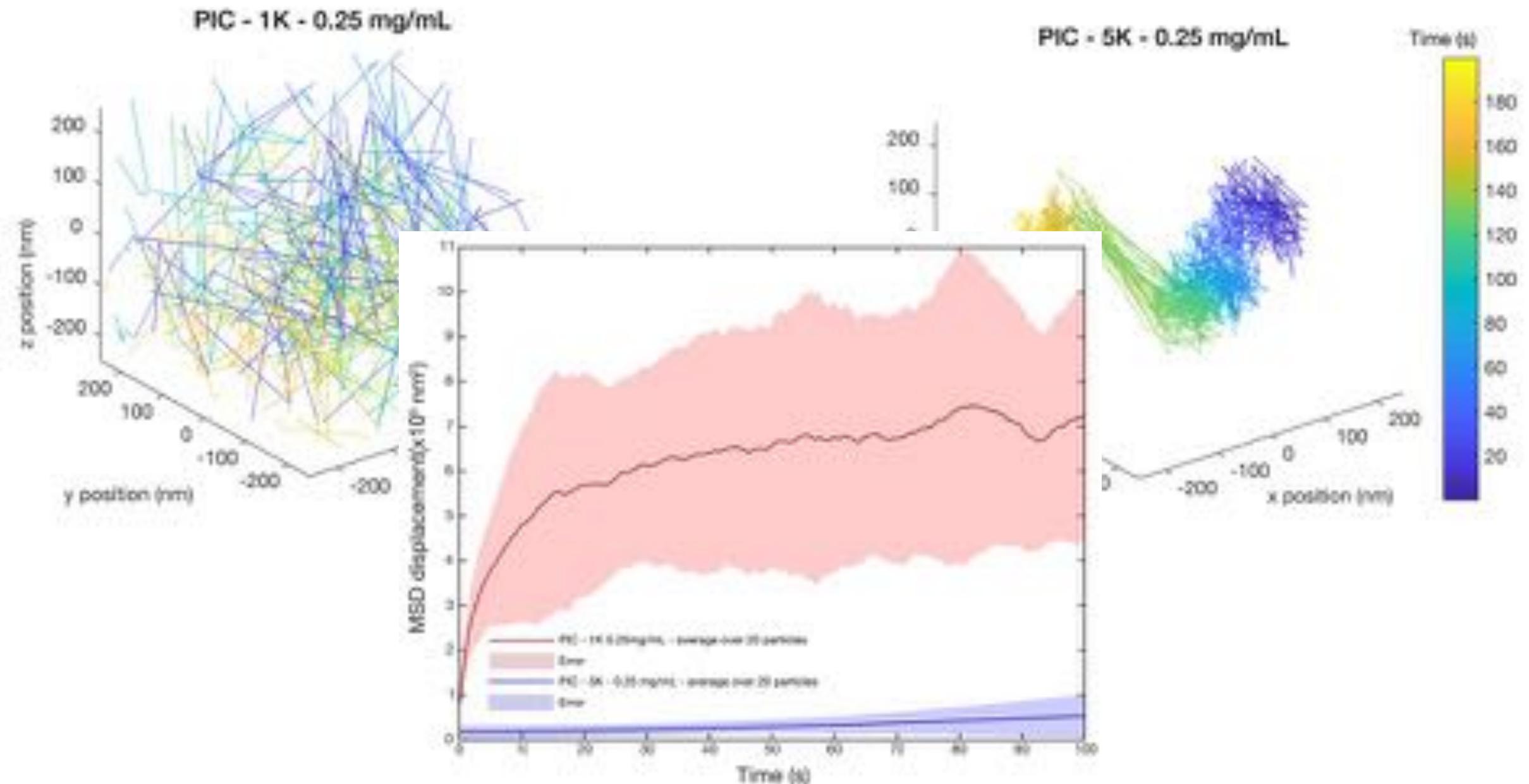
3D SPT in PIC hydrogel



3D SPT in PIC hydrogel

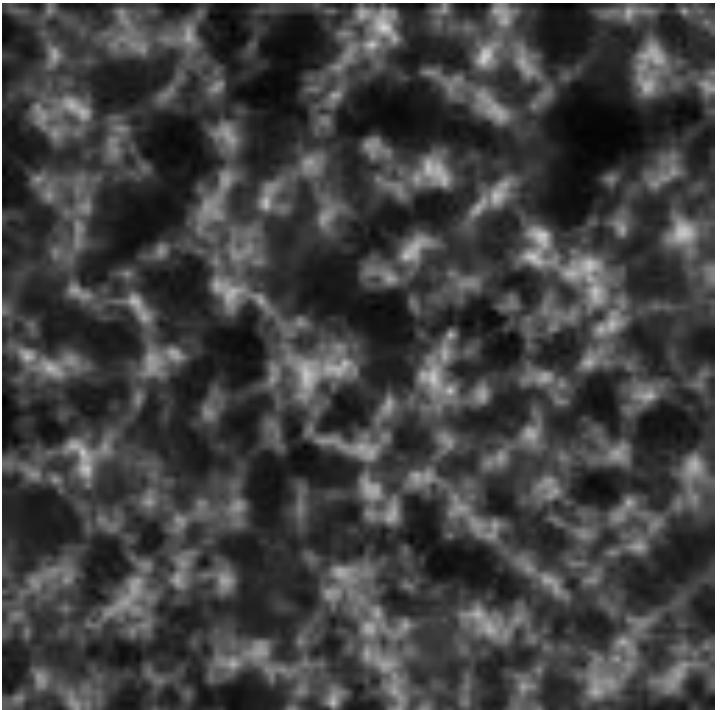


3D SPT in PIC hydrogel

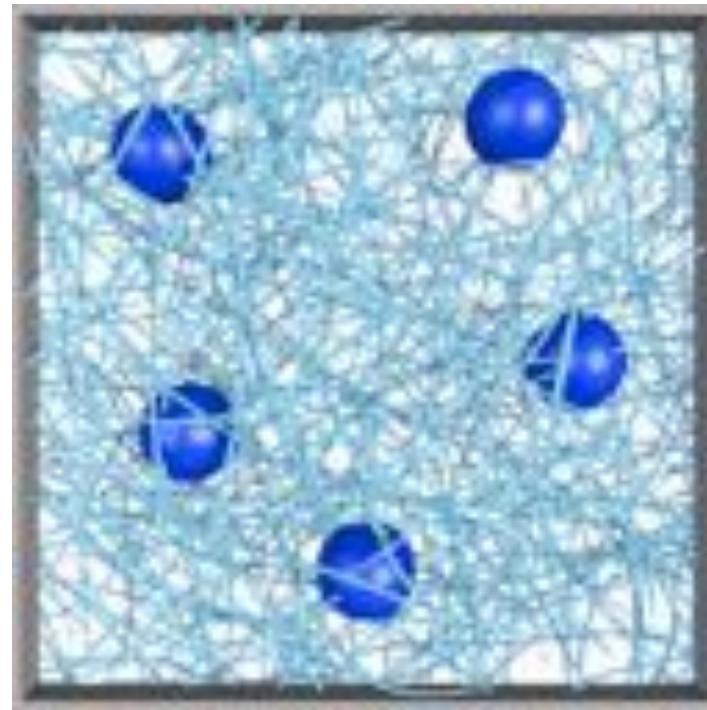


Material Characterization

Structure using advanced fluorescence microscopy

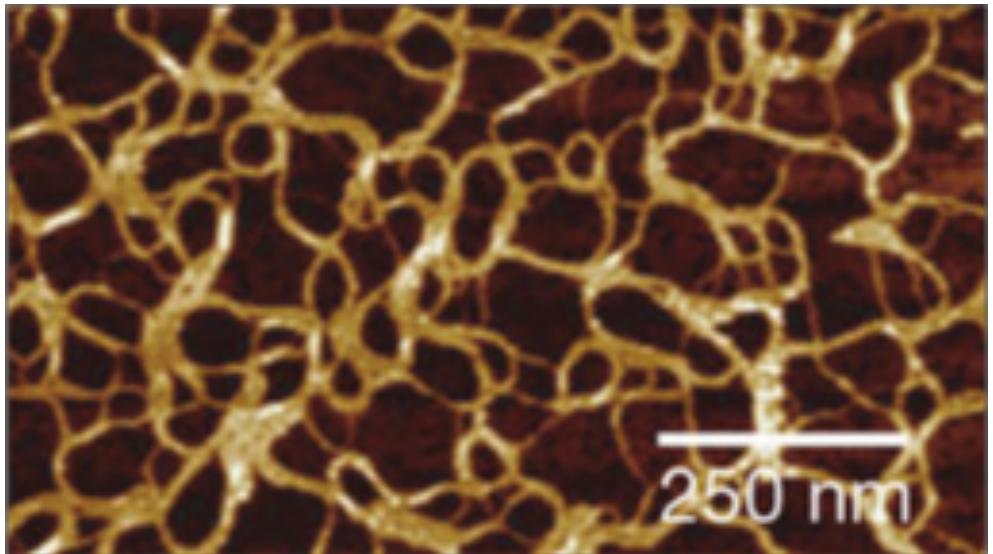


Local mechanical properties using 3D micro-rheology

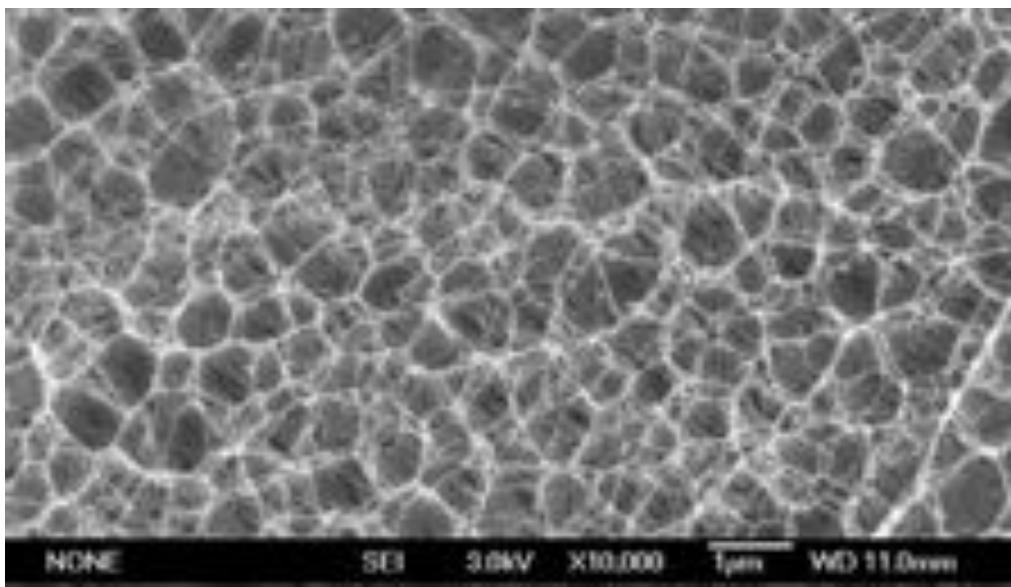


Structural Characterization of PIC-based hydrogels

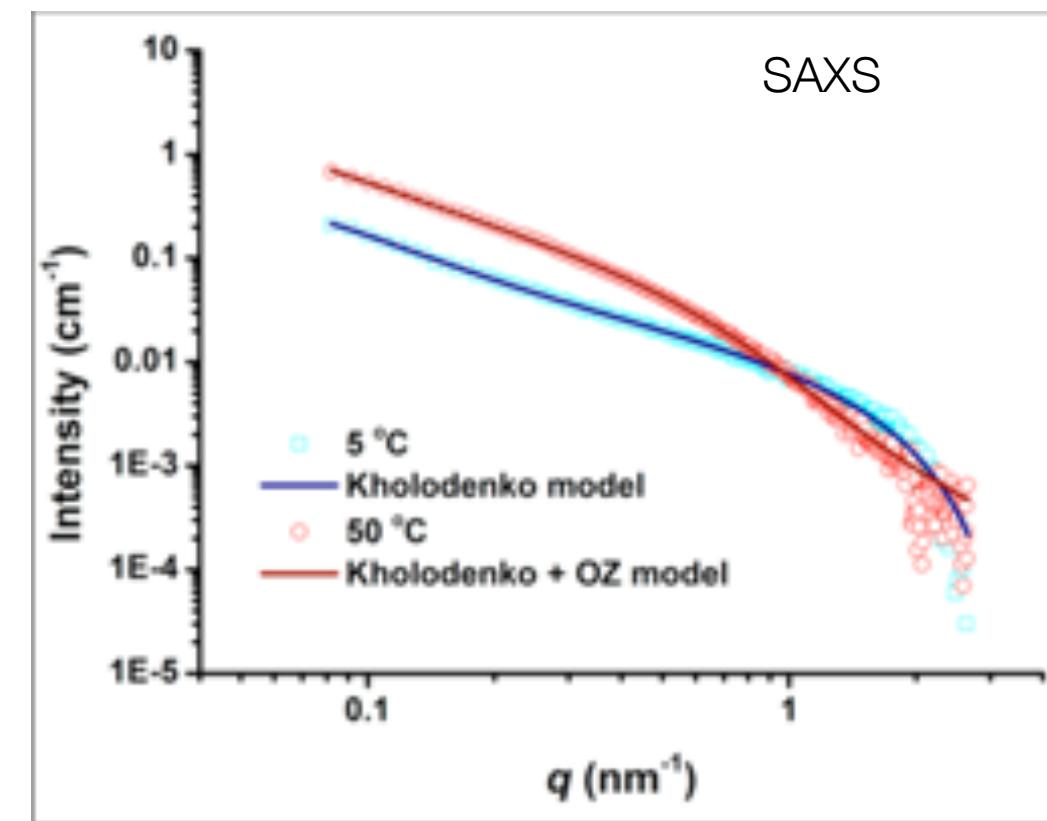
AFM



CryoEM



SAXS



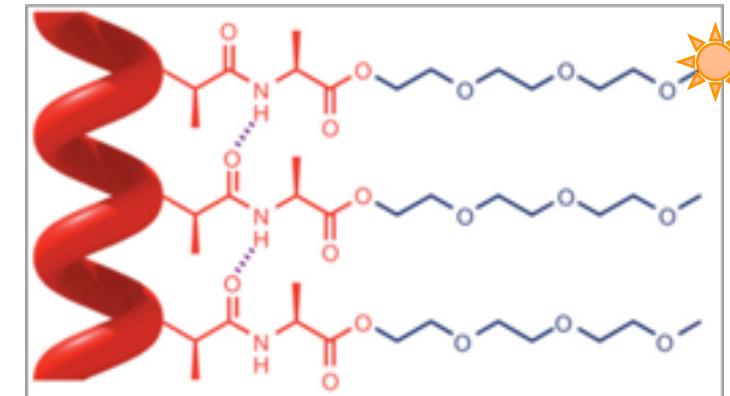
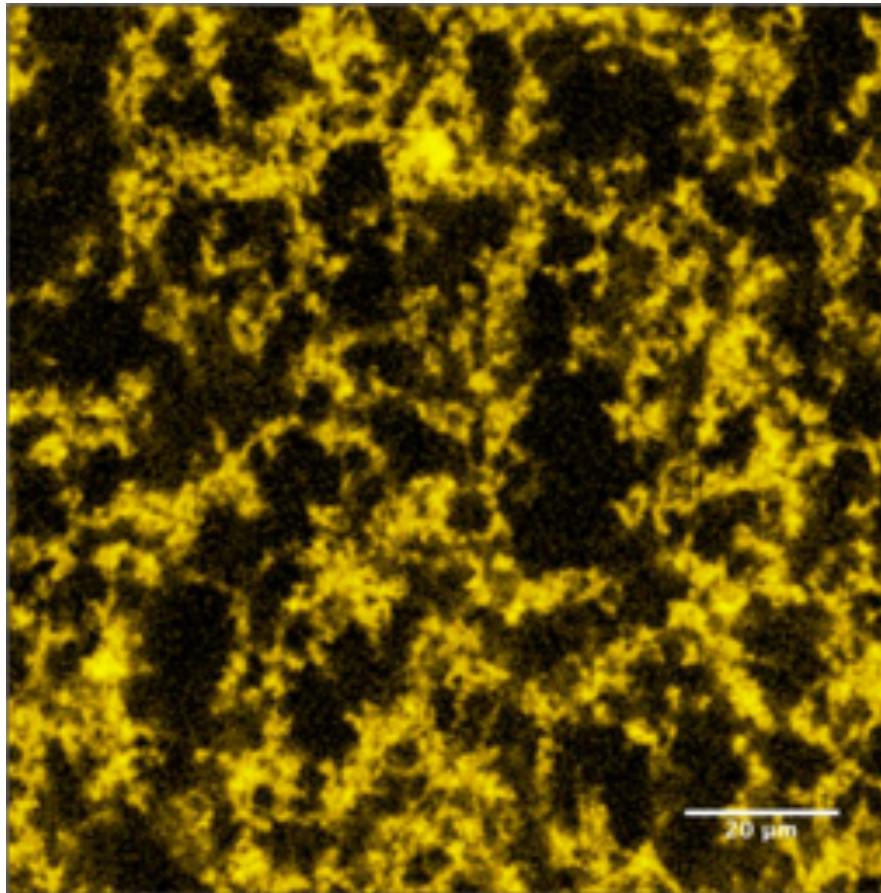
PIC	L (nm)	l_p (nm)	R (nm)	ξ_{OZ} (nm) ^b	R_B (nm)
P1a	55	13 ± 7	1.1 ± 0.1	$>80^{\text{c}}$	5.0 ± 0.2
P1b	77	10 ± 1	1.1 ± 0.1	$>80^{\text{c}}$	4.7 ± 0.2
P1c	110	9 ± 2	1.2 ± 0.1	$>80^{\text{c}}$	3.3 ± 0.1
P1b	134	9 ± 1	1.1 ± 0.1	68	3.0 ± 0.1
P1e	160	12 ± 2	1.1 ± 0.1	30	3.0 ± 0.2

Nature, 493, 651-655 (2014), doi: 10.1038/nature11839

Nature Communications, 5, 5808 (2014), doi: 10.1038/ncomms6808

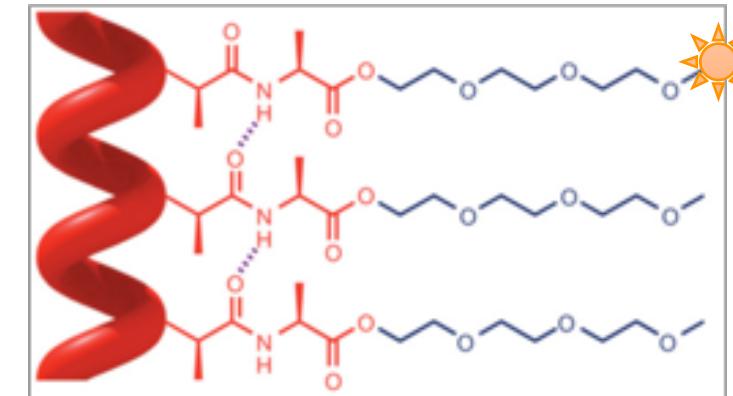
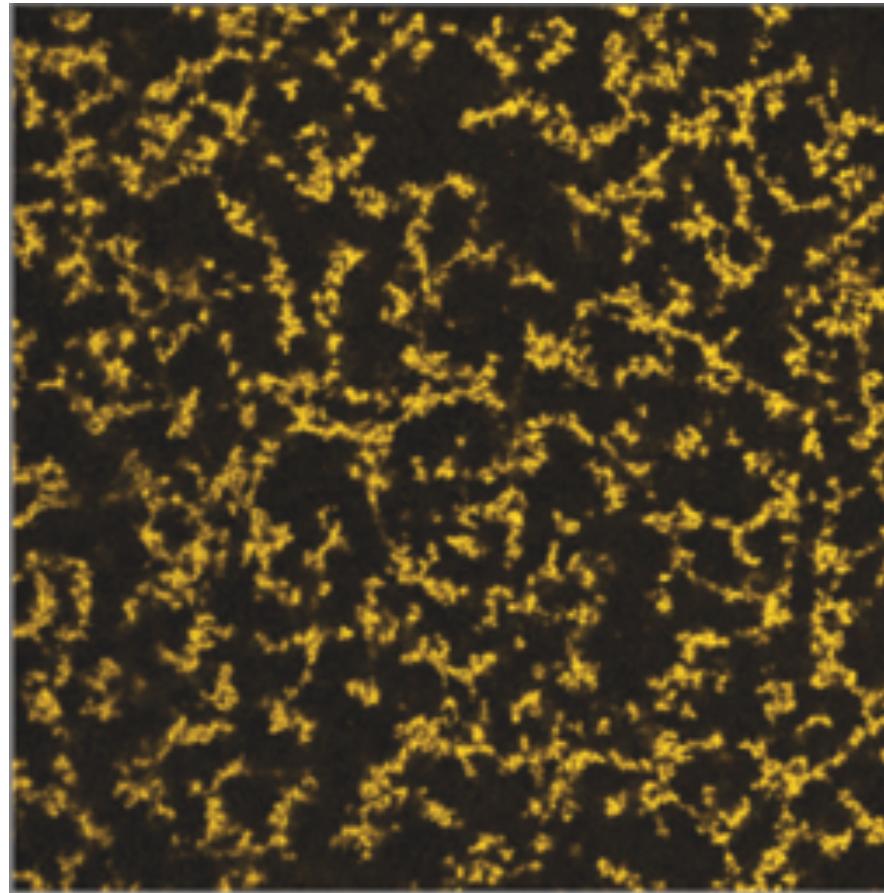
Structural Characterization of PIC-based hydrogels

Fluorescence Microscopy



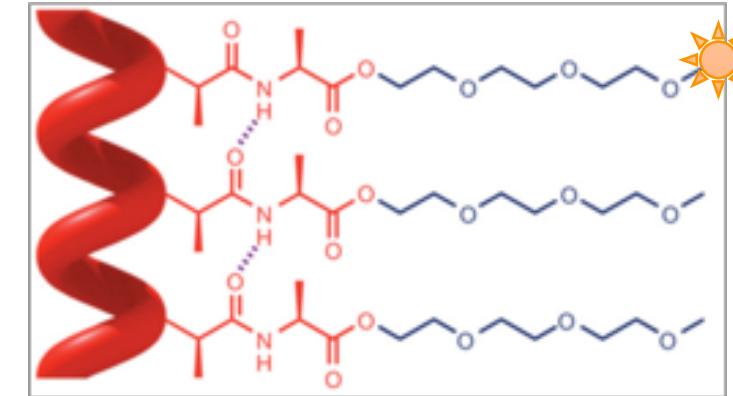
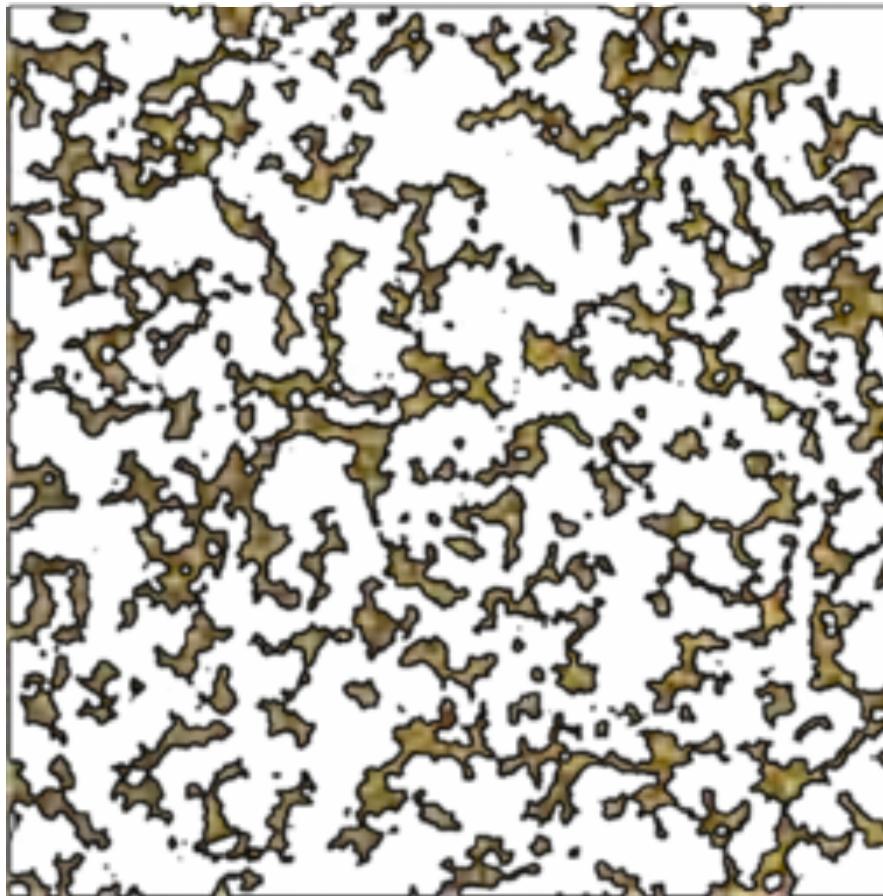
Structural Characterization of PIC-based hydrogels

Fluorescence Microscopy

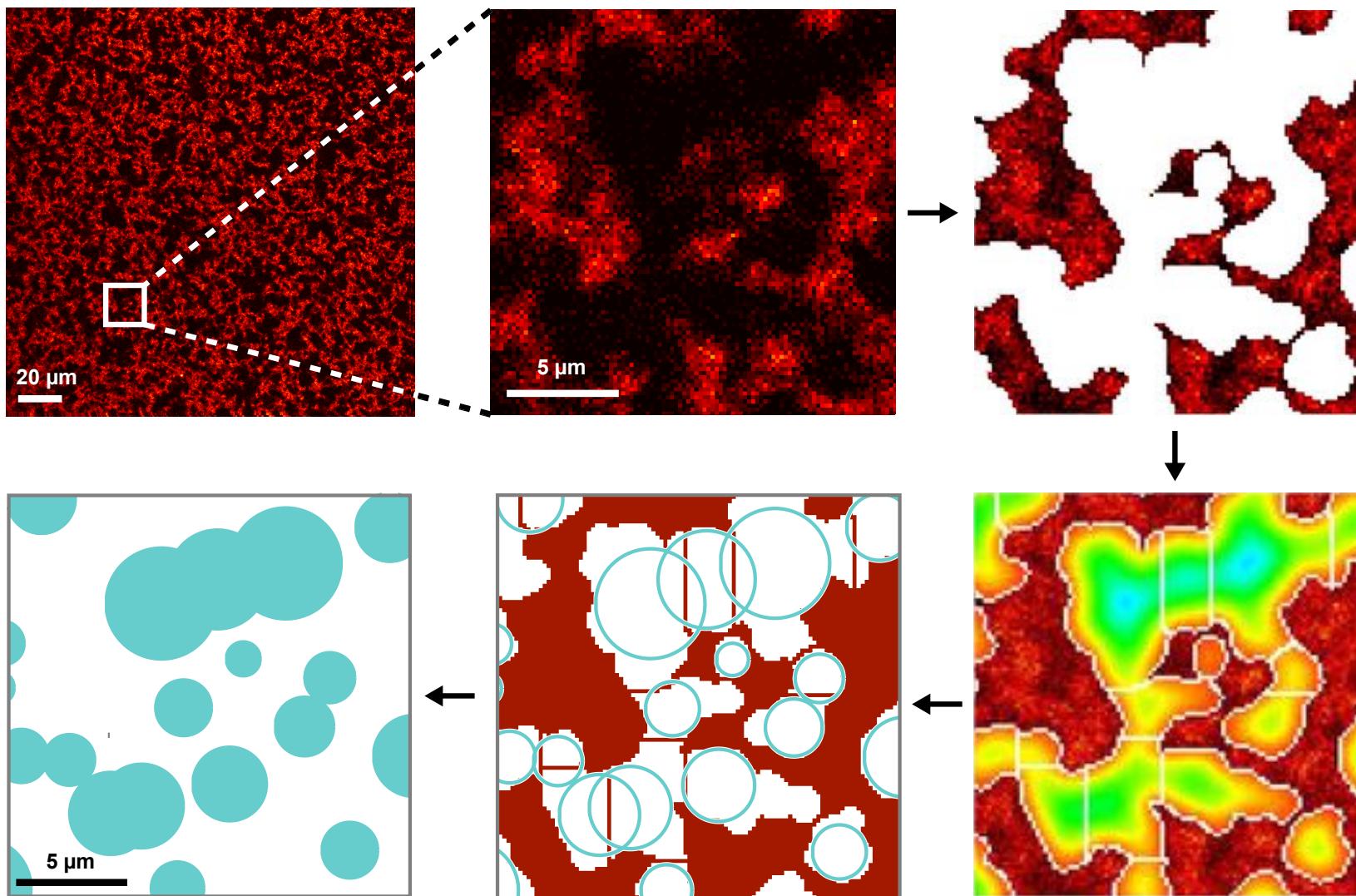


Structural Characterization of PIC-based hydrogels

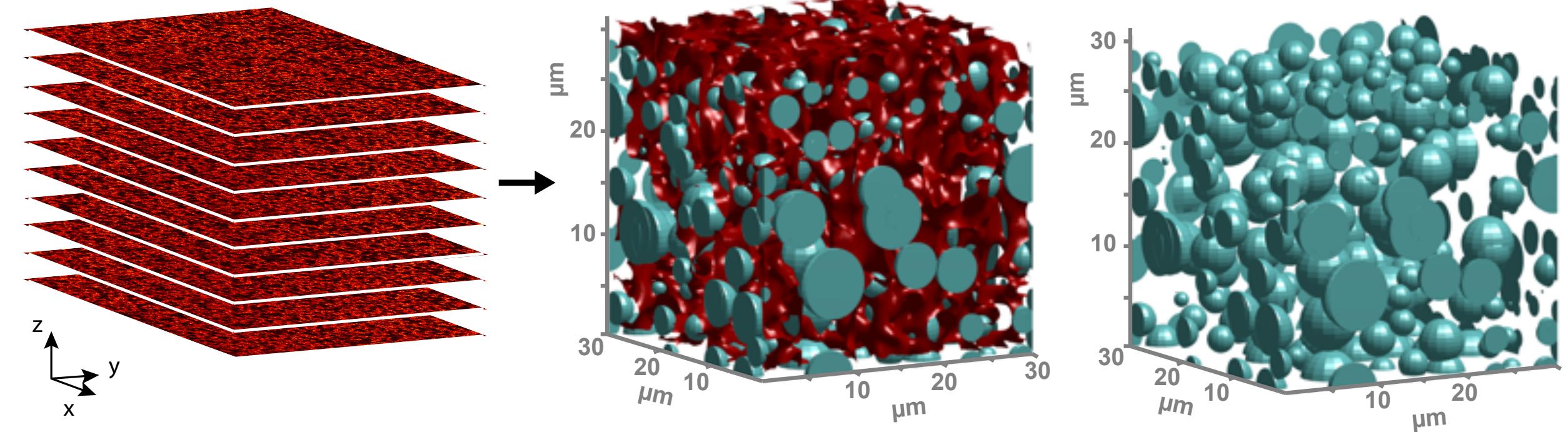
Fluorescence Microscopy



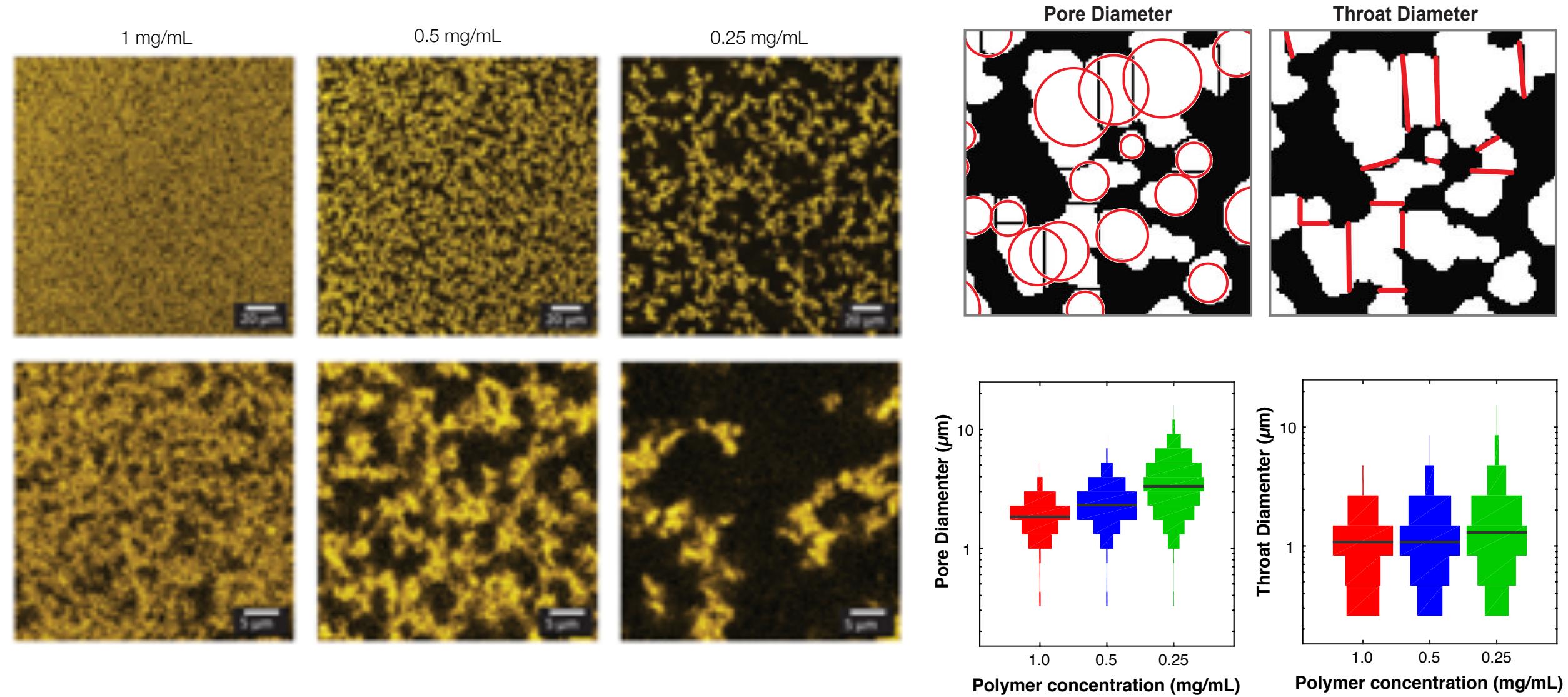
Structural Characterization of PIC-based hydrogels



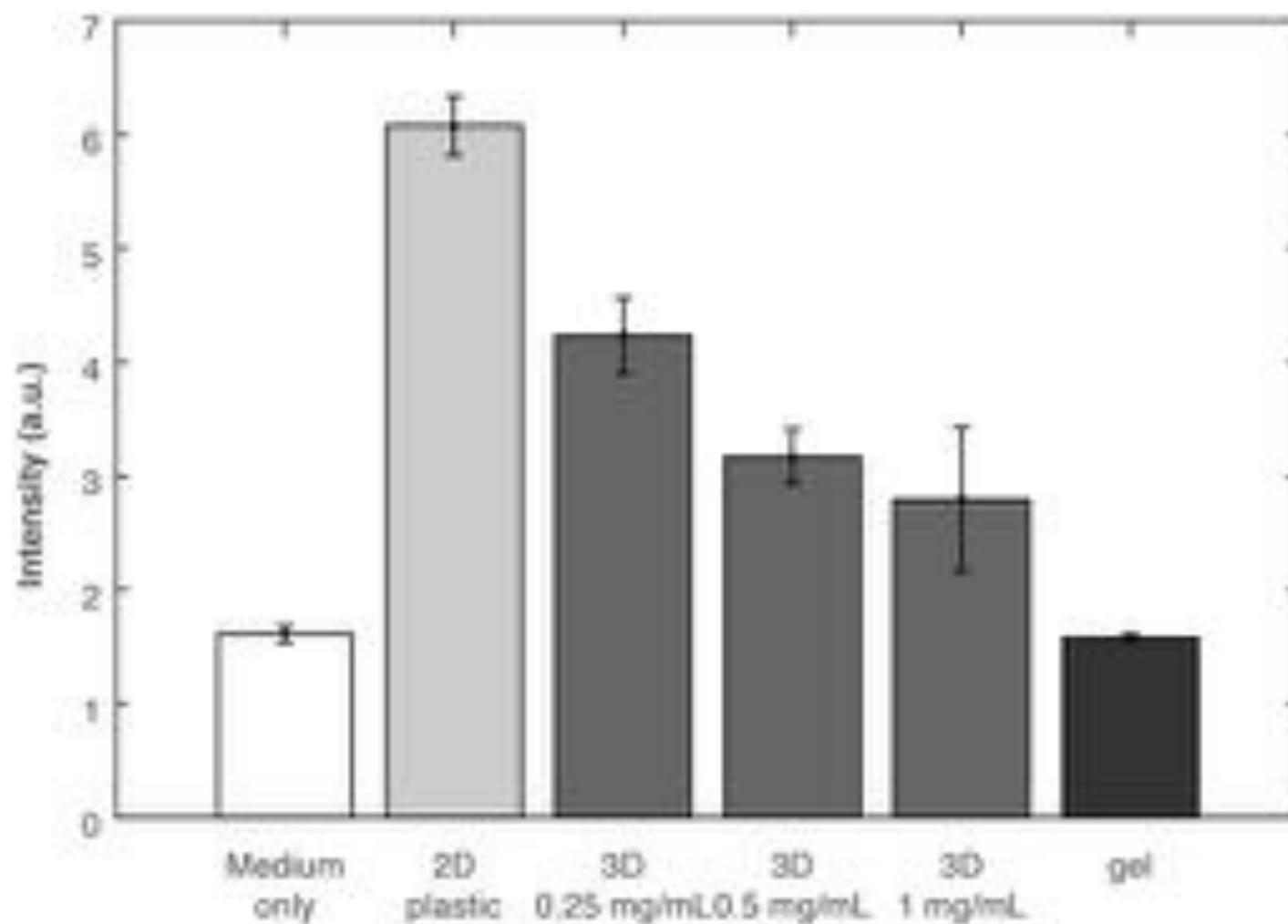
Structural Characterization of PIC-based hydrogels in 3D



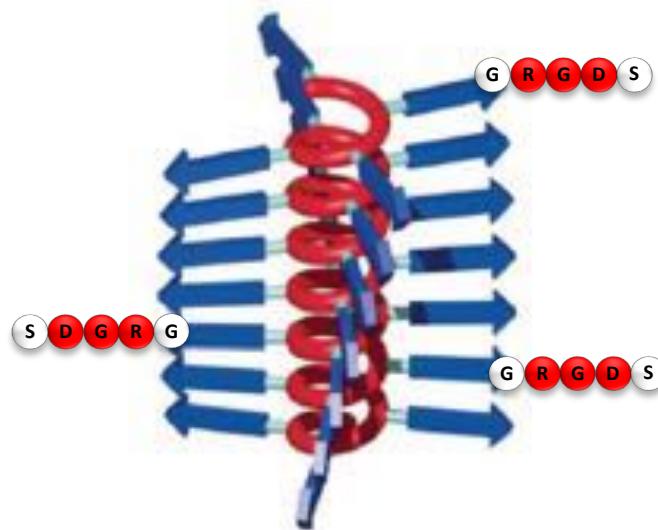
Structural Characterization of PIC-based hydrogels



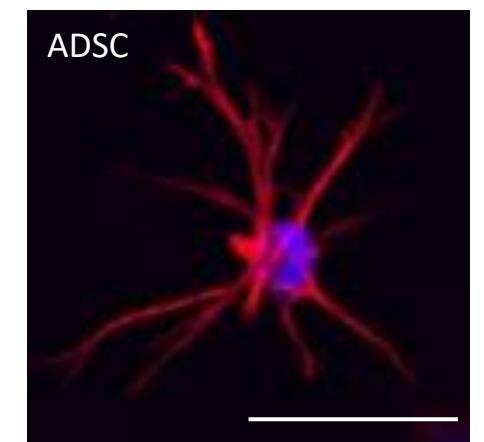
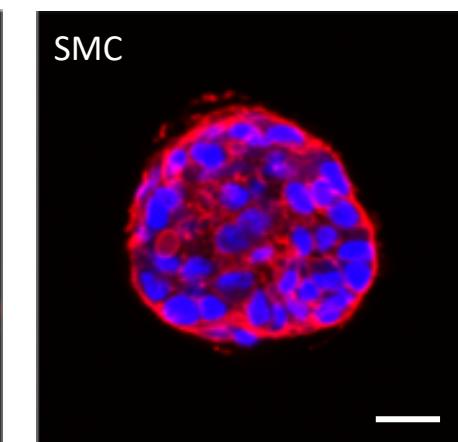
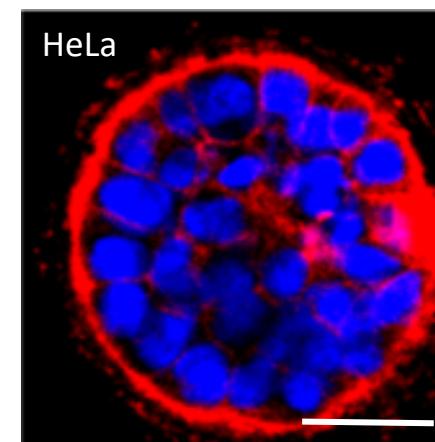
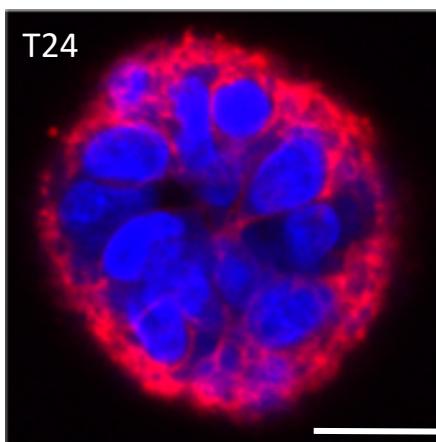
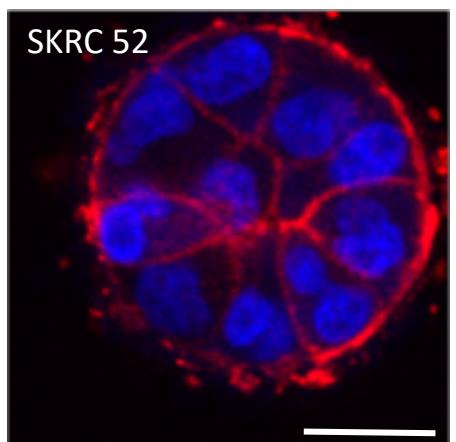
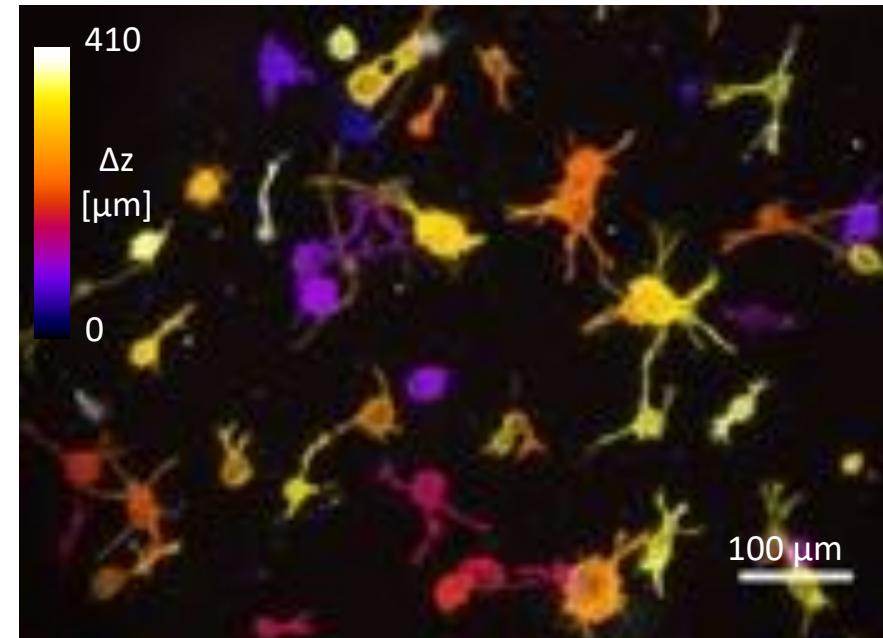
Hydrogel structure and cell proliferation



Minimalist matrix: Polyisocyanopeptides (PIC)

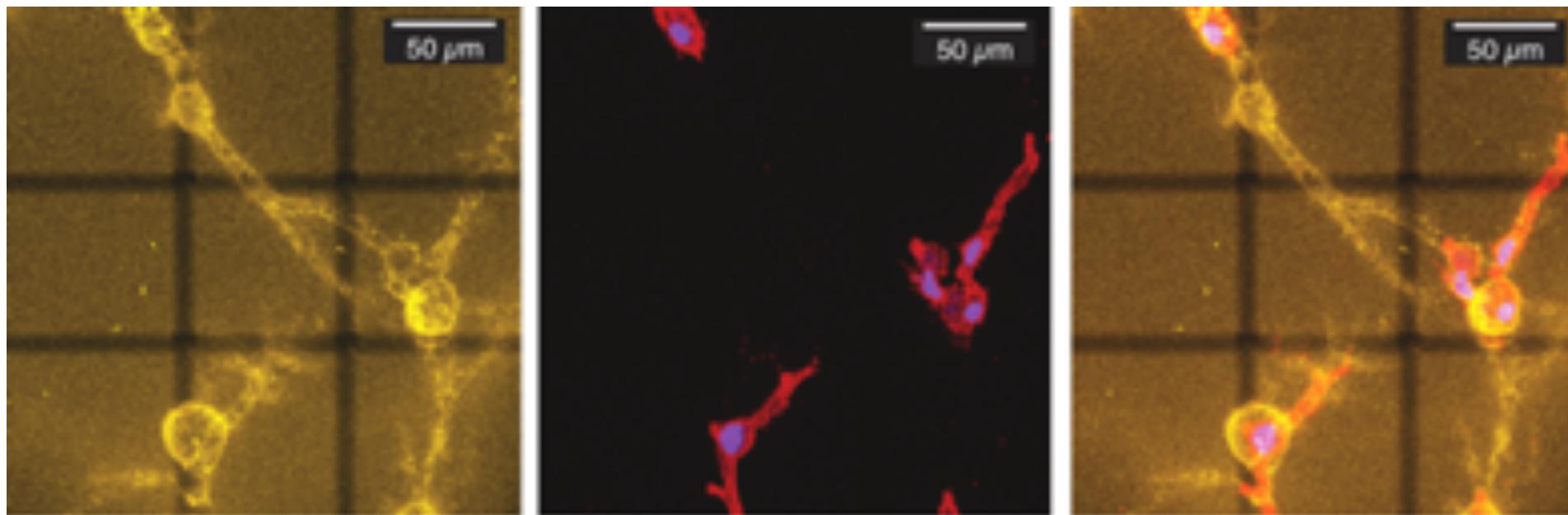
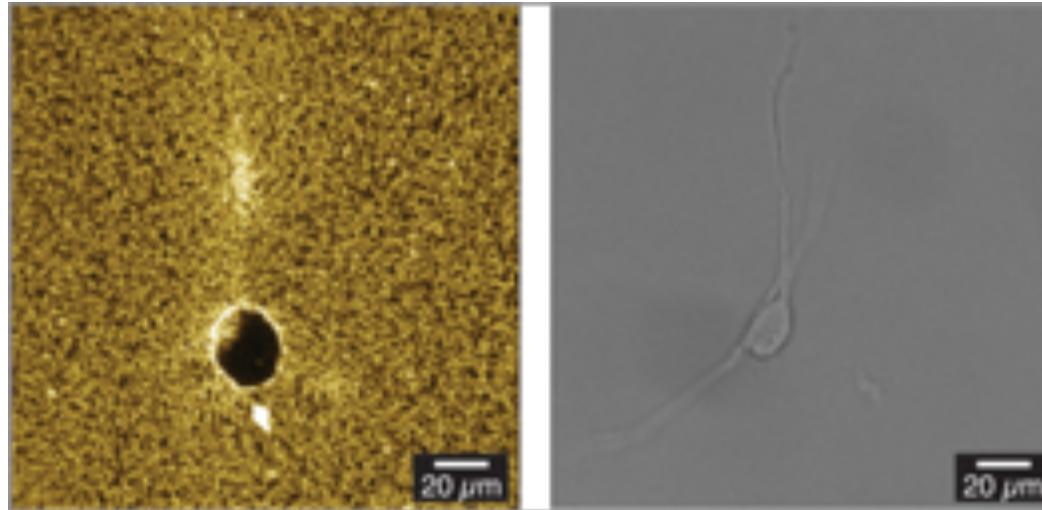


Different cell types → Different behavior

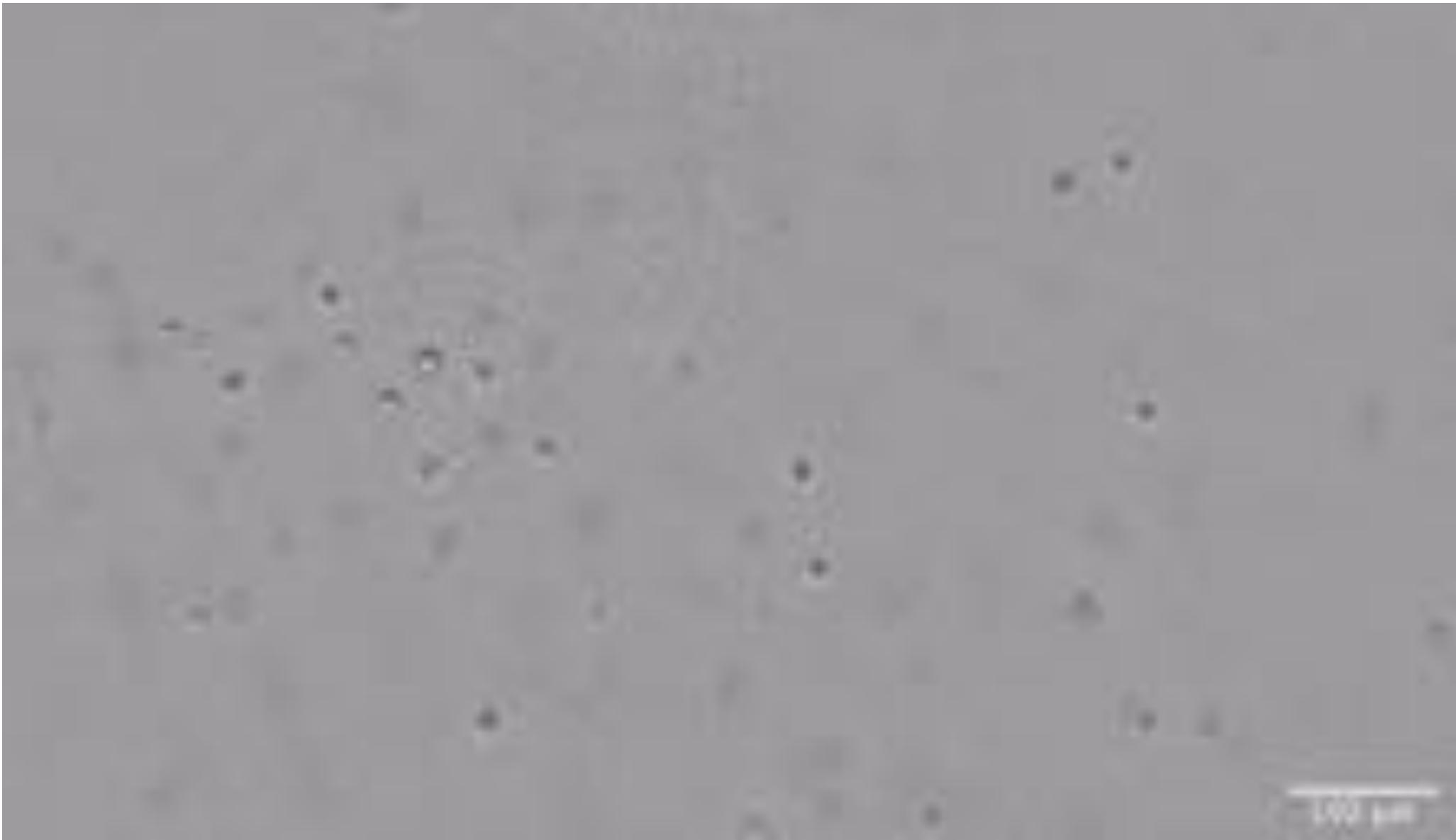


Phalloidin
DAPI

Matrix remodeling by human Adipose Stem Cells



PIC F-actin Nucleus





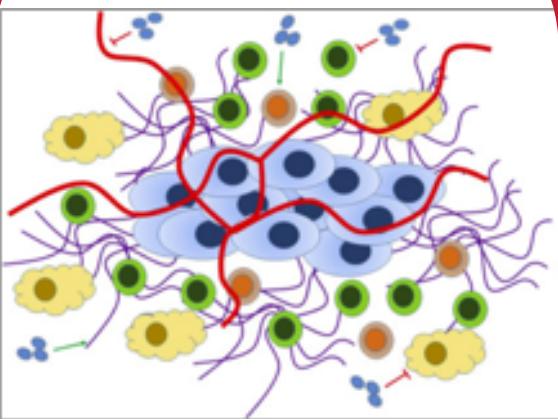
Materials in medicine

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- 2. Nanocarriers and Advanced cancer models*

Susana Rocha

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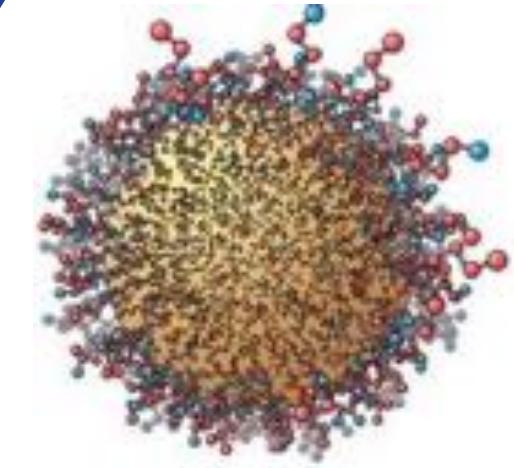
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Cancer

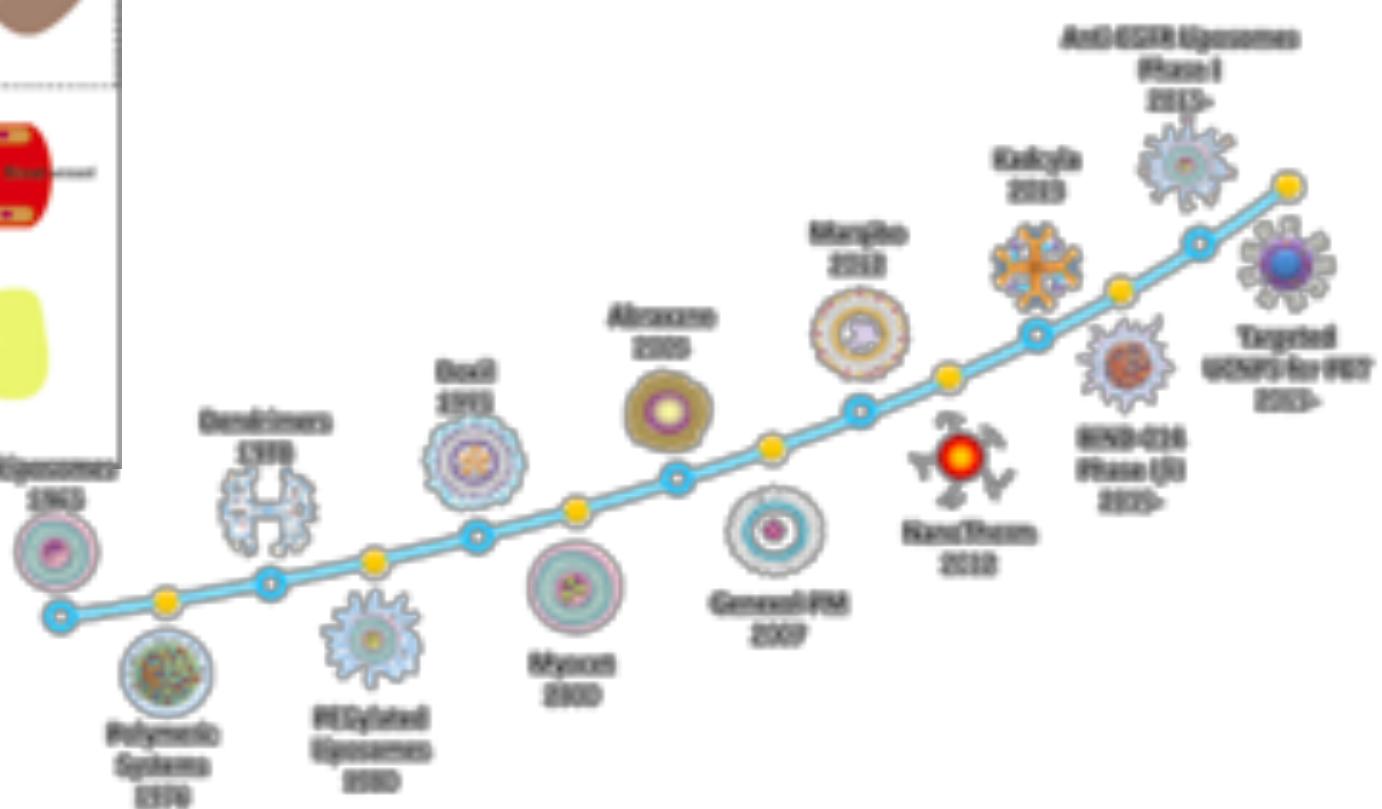
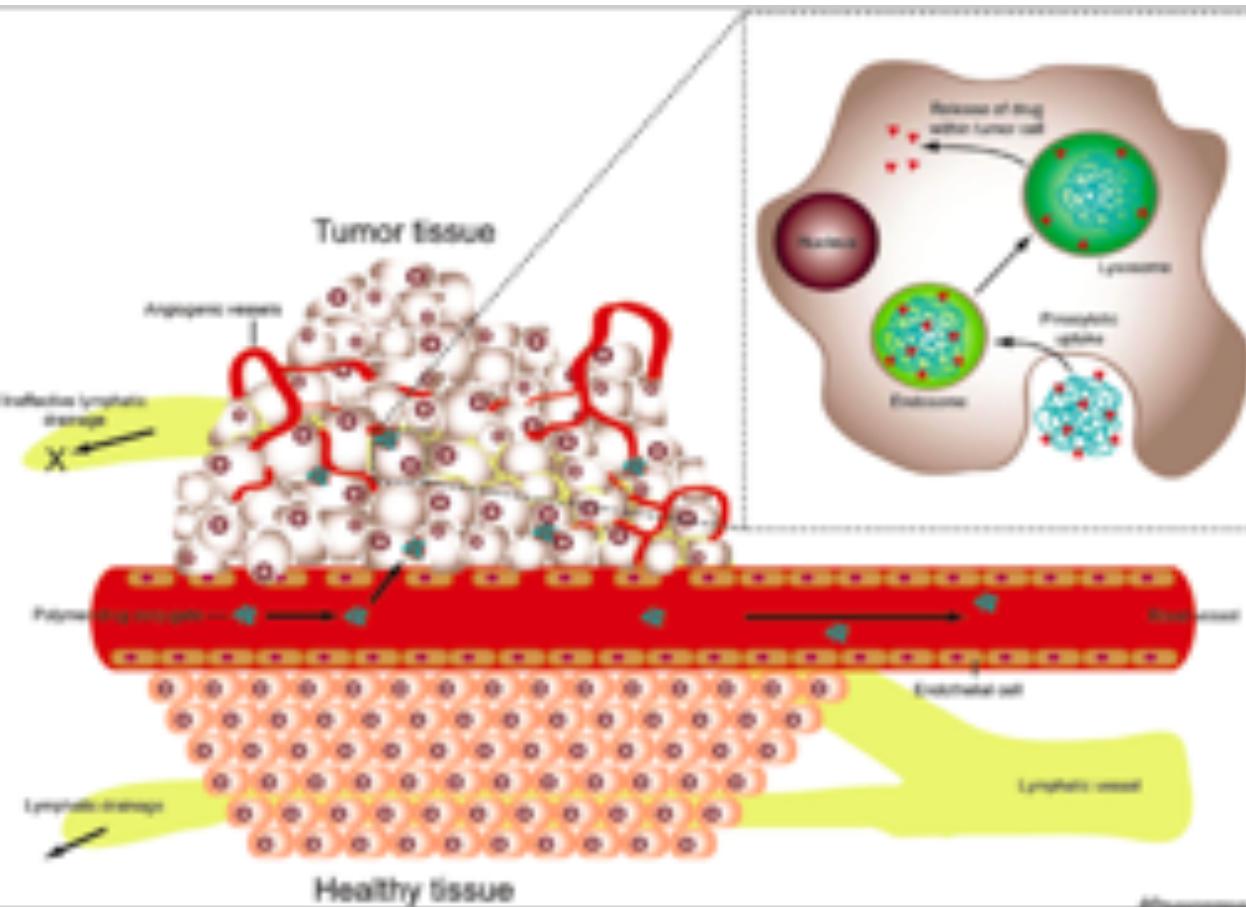


Chemotherapy

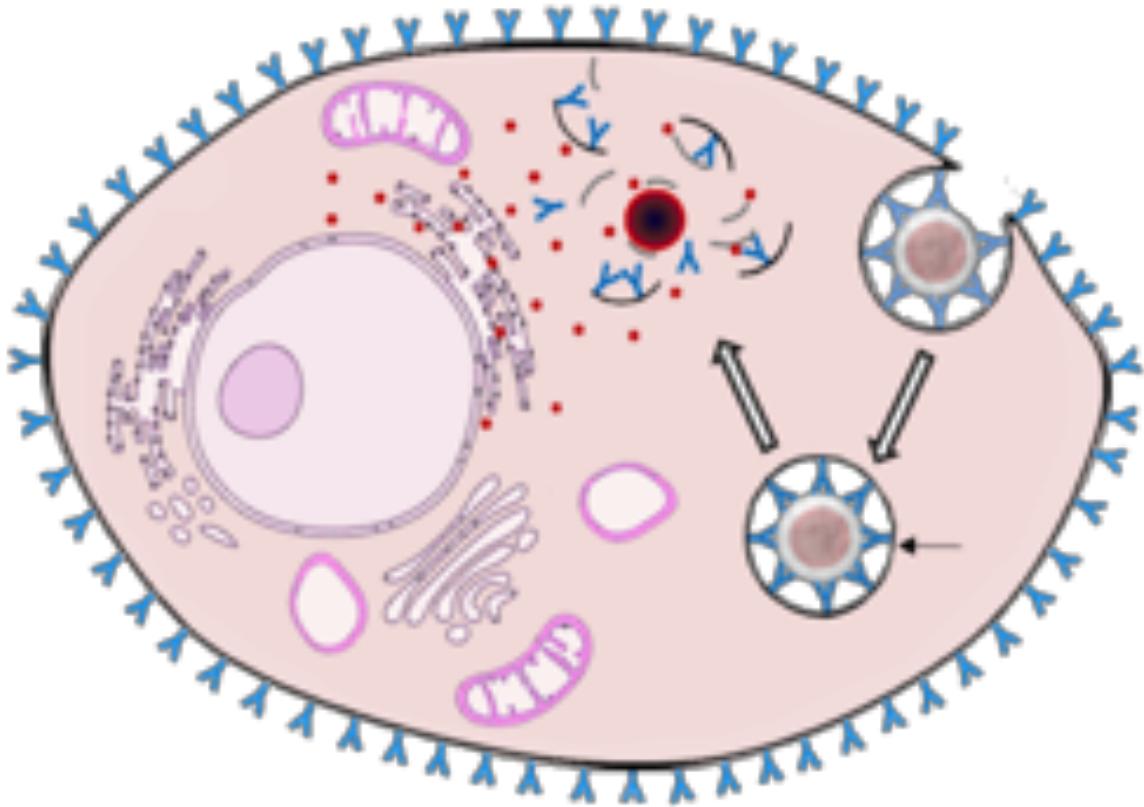


Nanocarriers

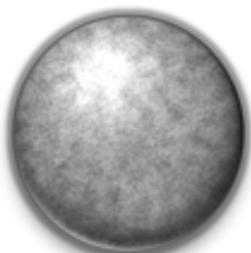
Development of nanomedicines



Our drug delivery system (DDS)

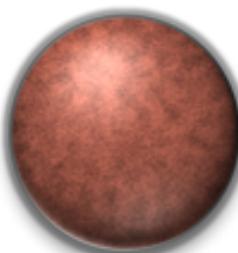


MSNP



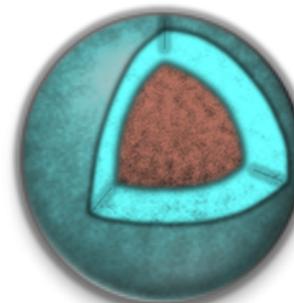
Dox
→

MSNP_Dox



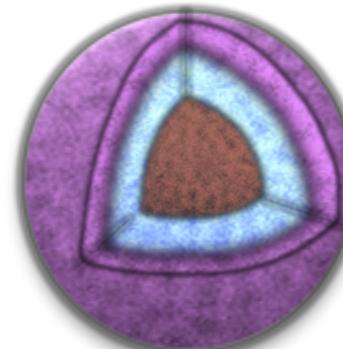
PEI
→

PEI-MSNP_Dox

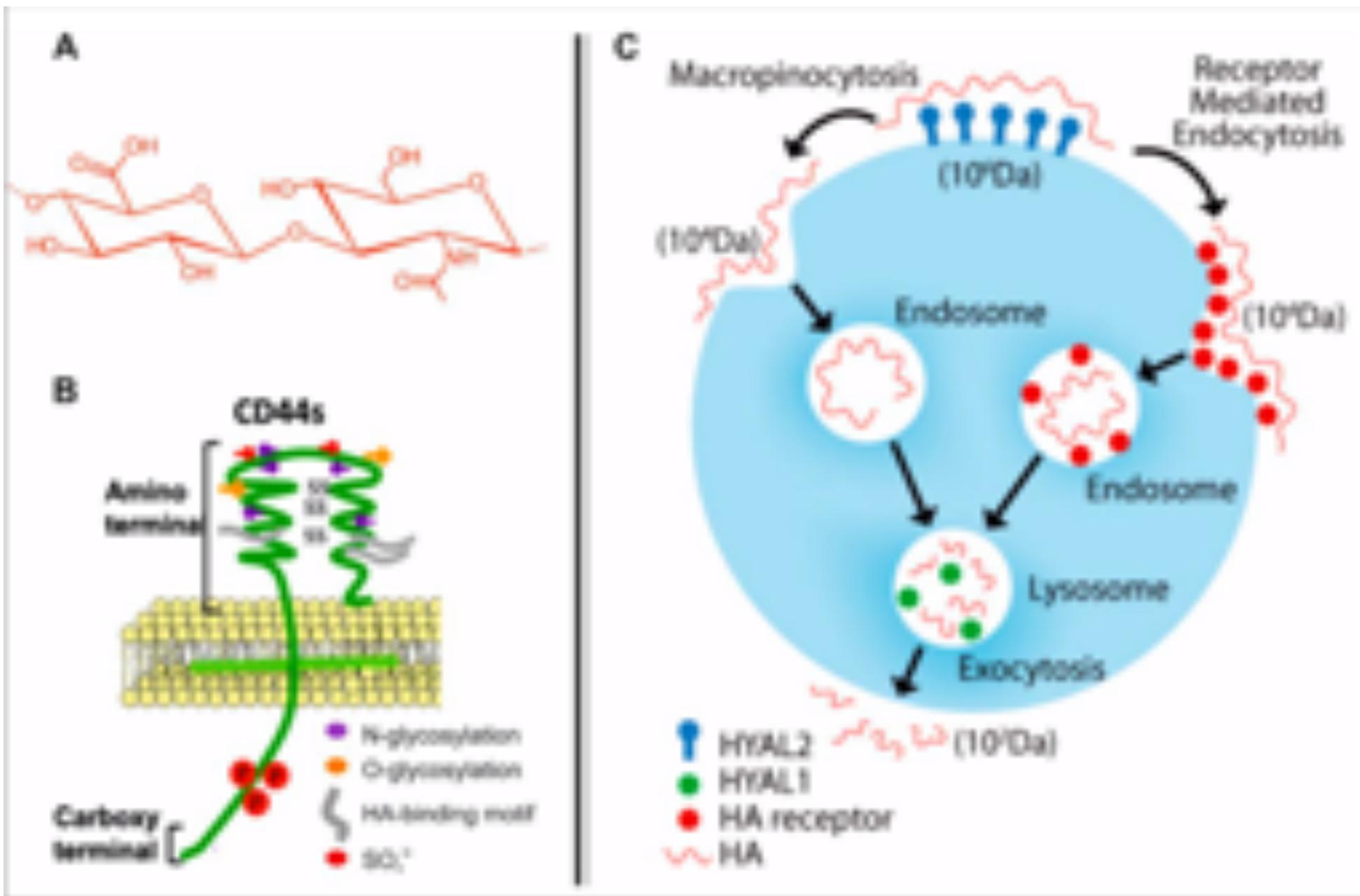


hyaluronic
acid
→

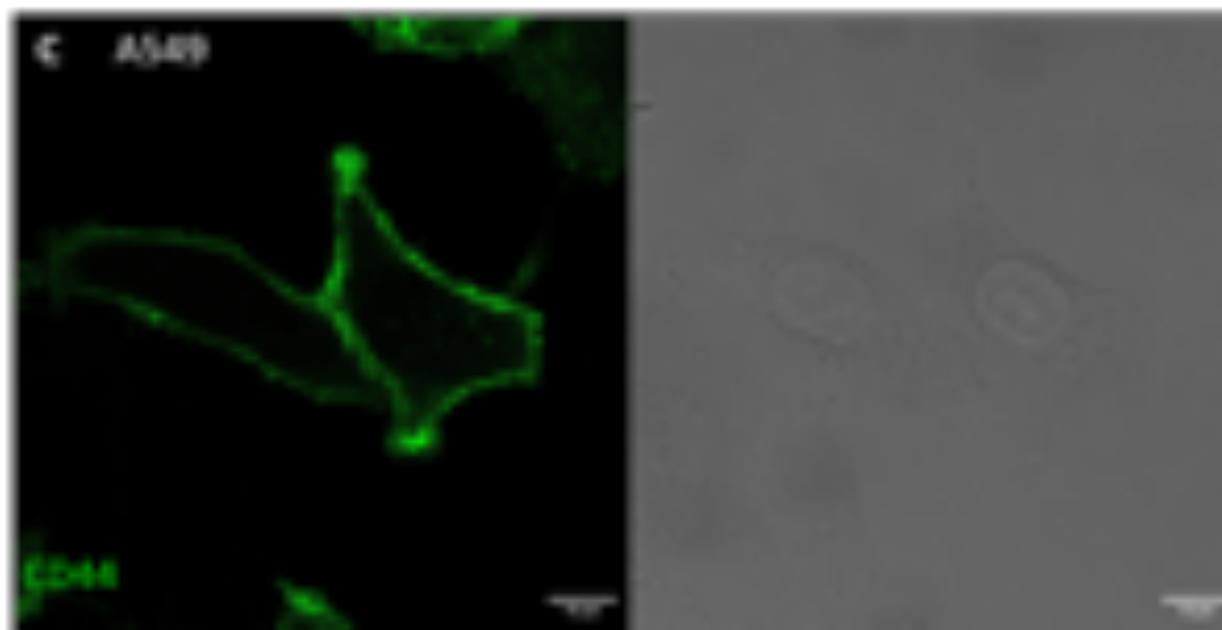
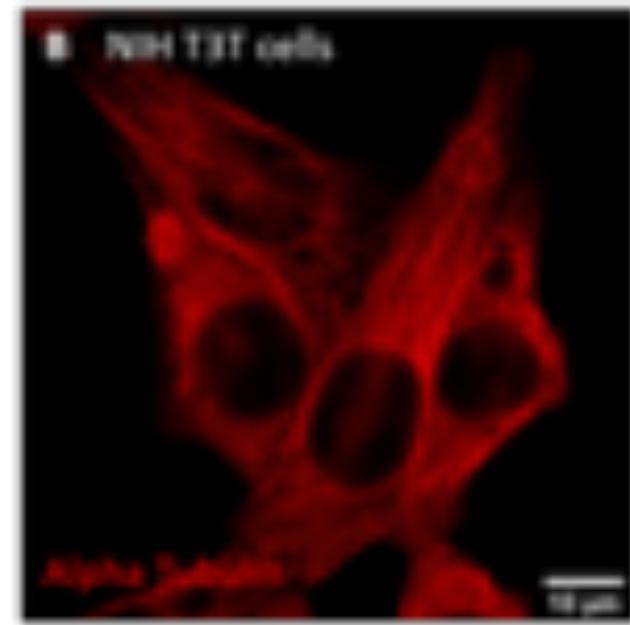
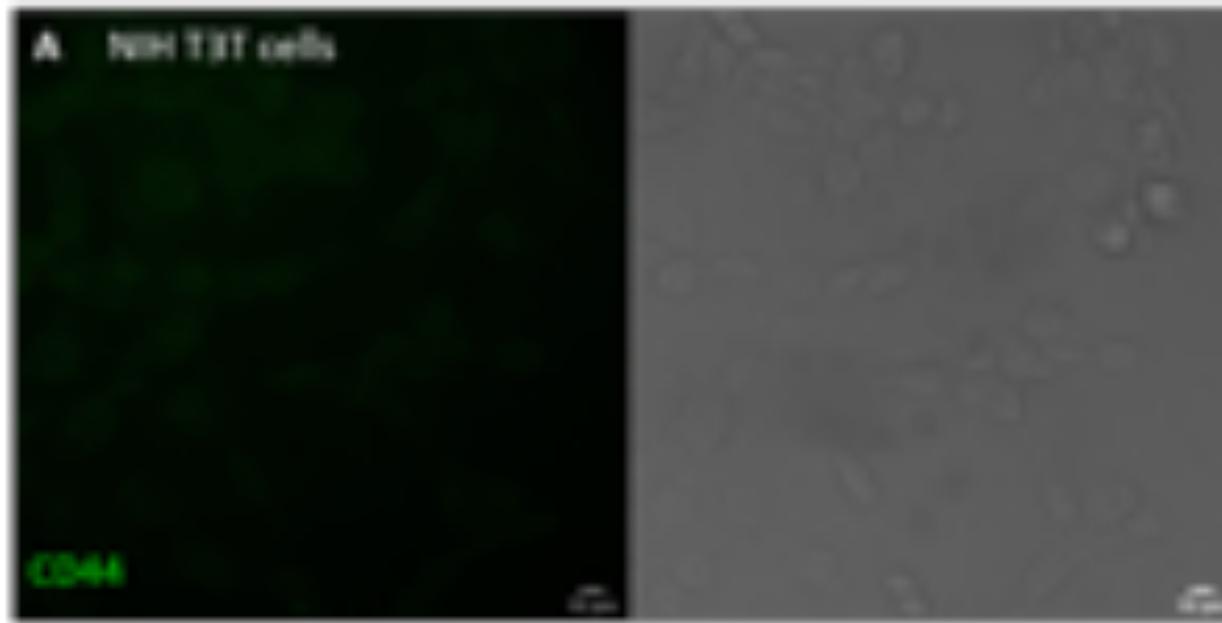
HAPEI-MSNP_Dox



Targeting CD44 with HA

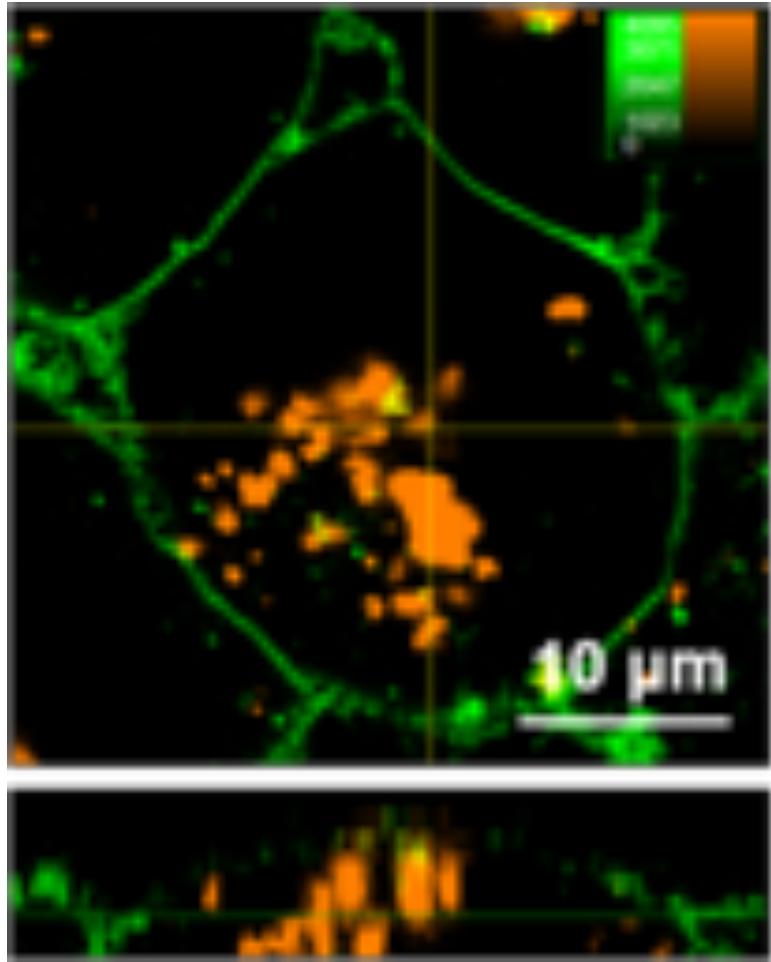


Cancer models (2D)

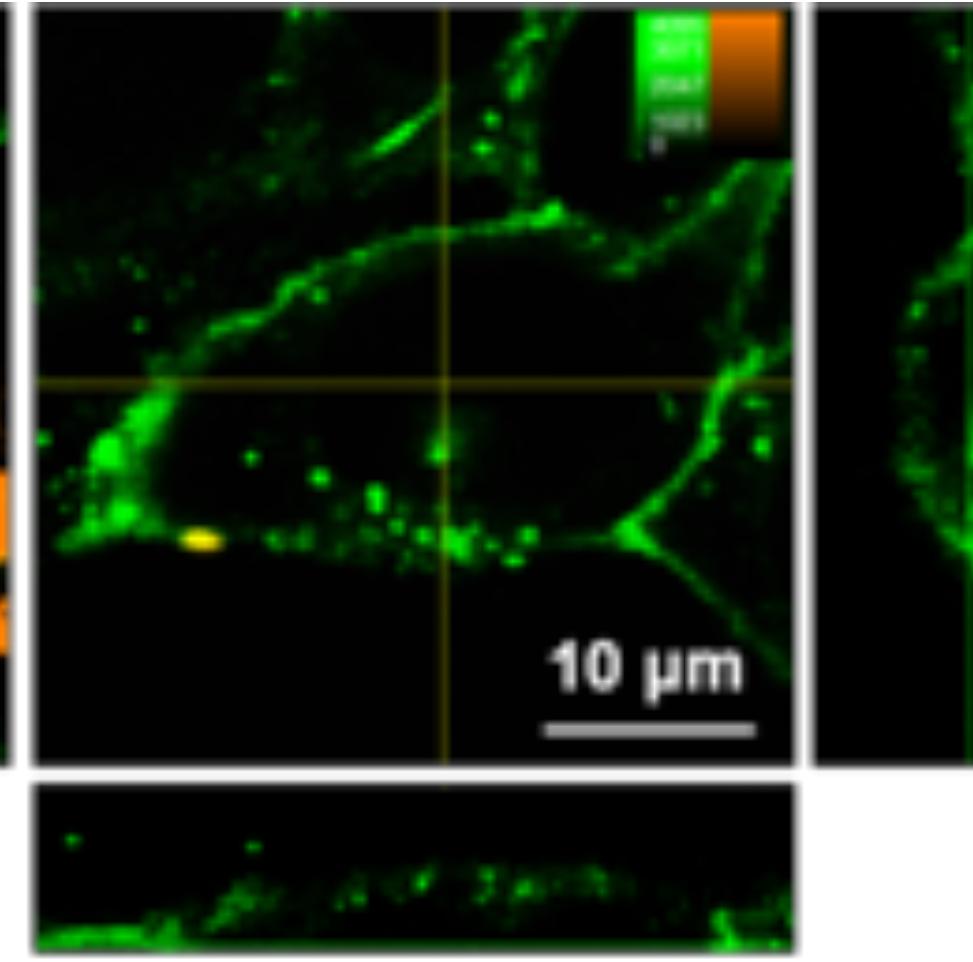


Specificity towards cancer cells

A549 (CD44+)

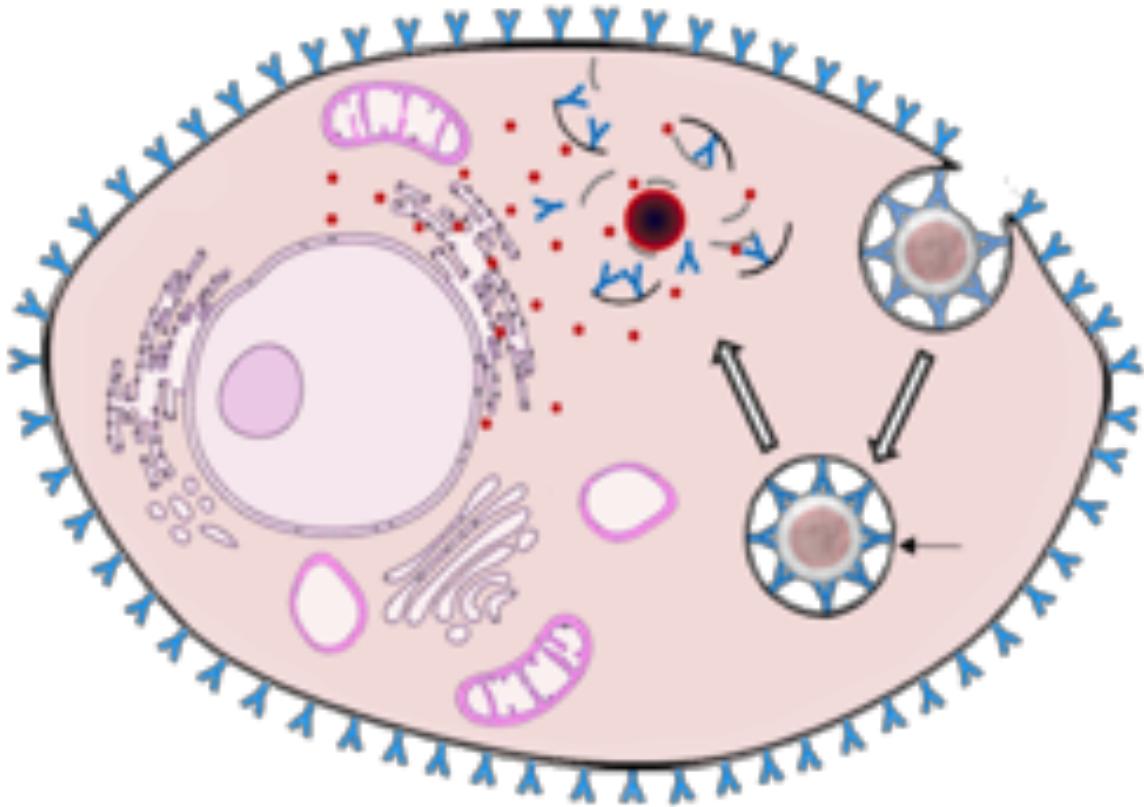


NIH 3T3 (CD44-)



Membrane Dye (DiO)
MSNPs

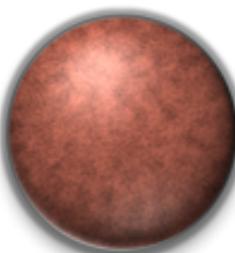
Our drug delivery system (DDS)



MSNP

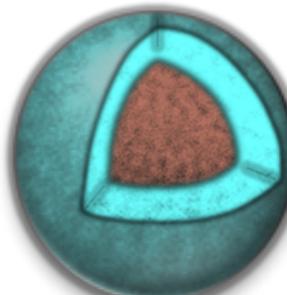


MSNP_Dox



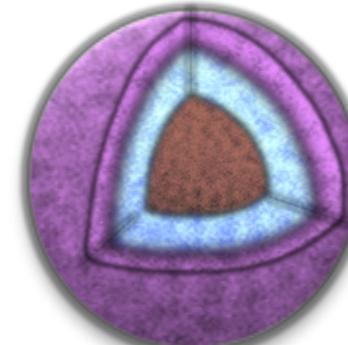
Dox
→

PEI-MSNP_Dox



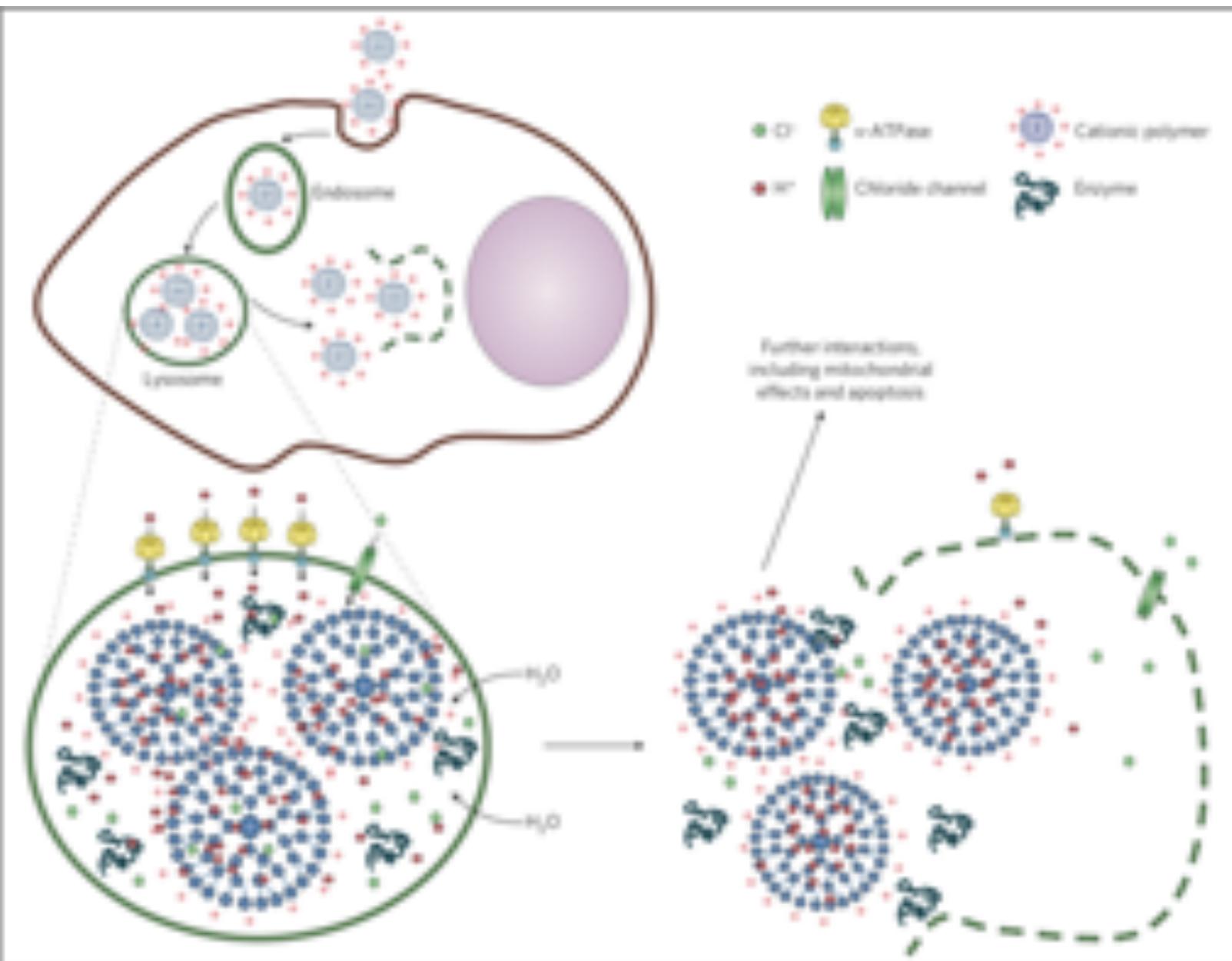
PEI
→

HAPEI-MSNP_Dox

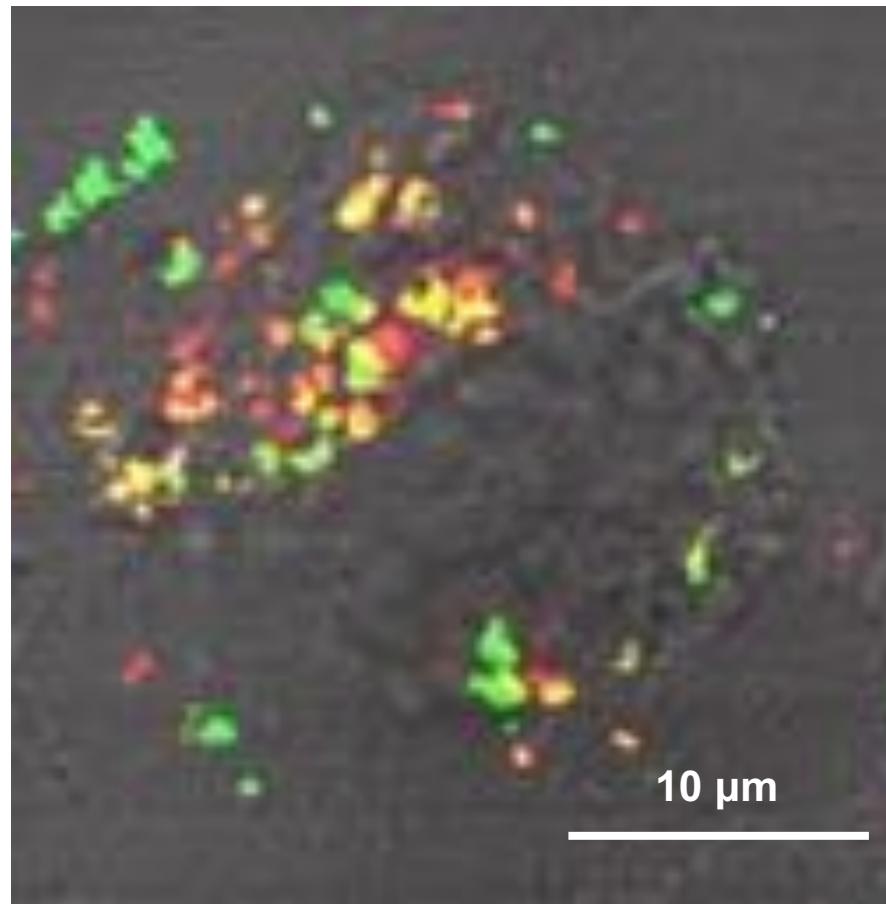


hyaluronic
acid
→

Endosomal escape – Proton sponge effect



Cellular uptake and vesicle trafficking

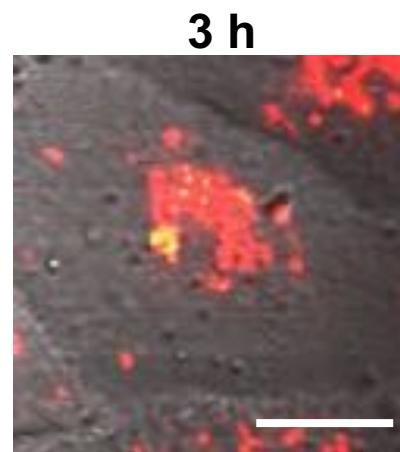


LysoTracker Red
MSNPs

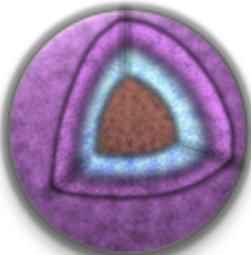
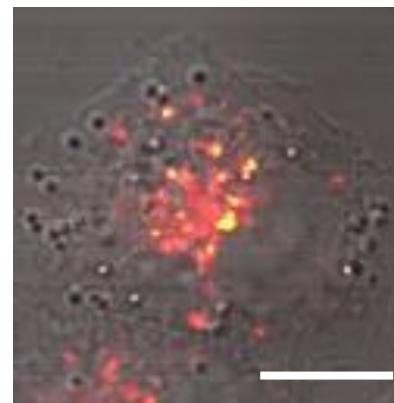
Cellular uptake and vesicle trafficking



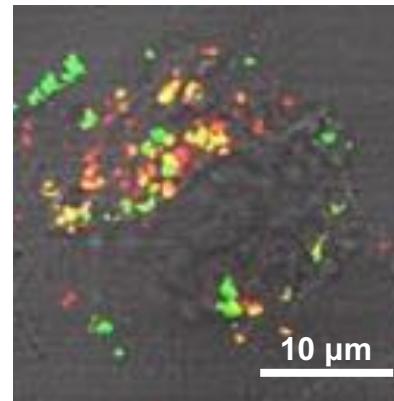
MSNPs_FITC



PEI-MSNPs_FITC

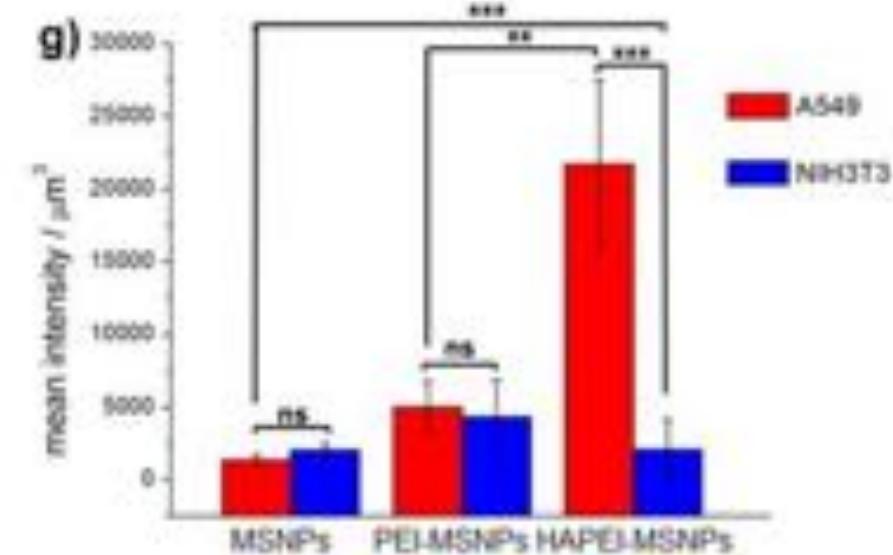
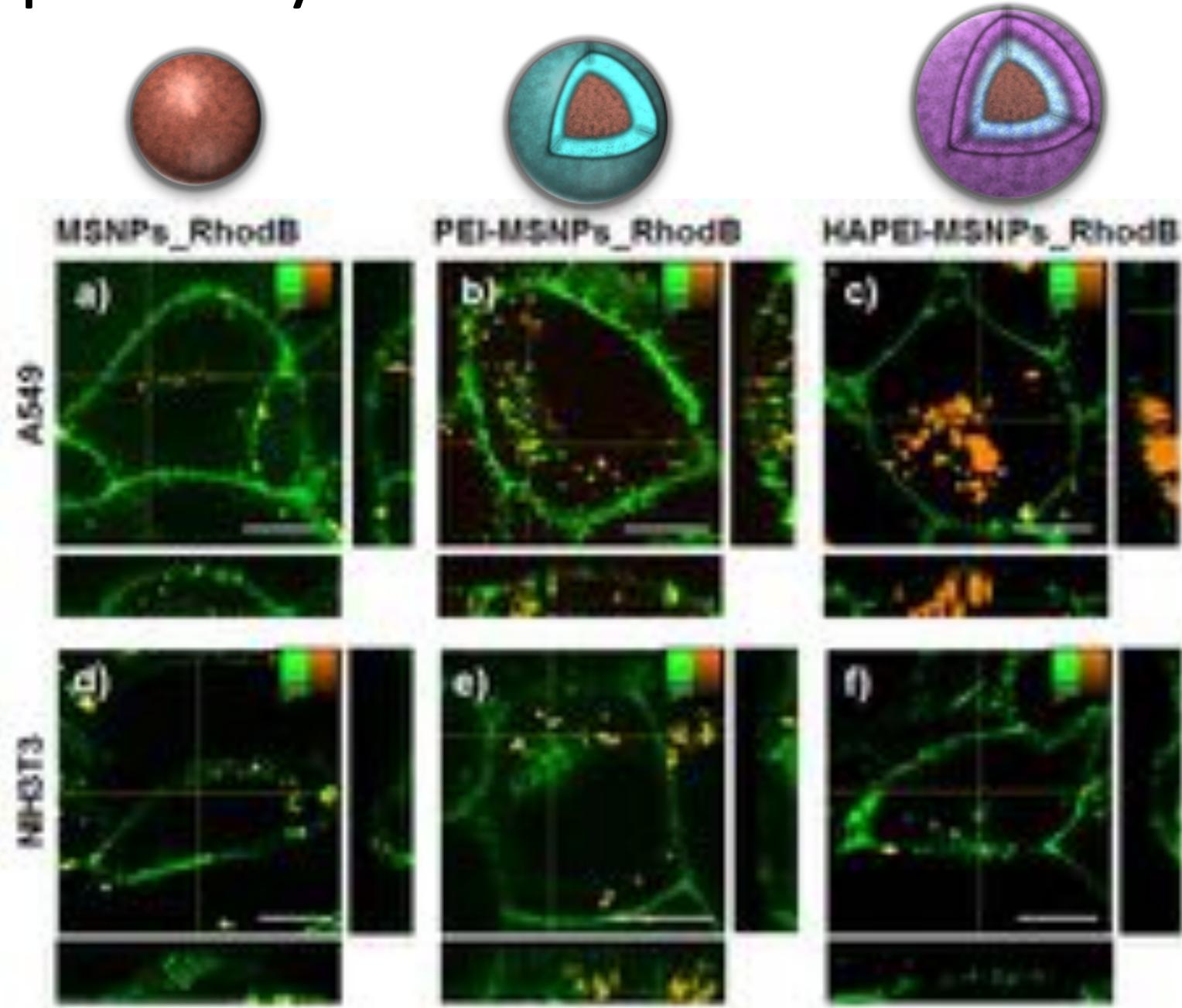


HAPEI-MSNPs_FITC



LysoTracker Red
MSNPs

Specificity to CD44+ cells

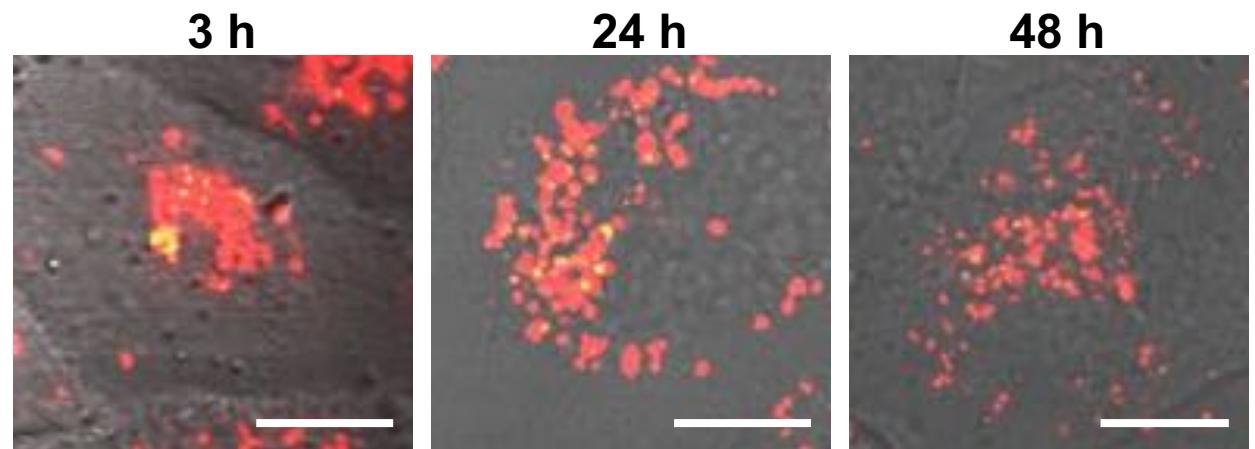


Membrane Dye (DiO)
MSNPs

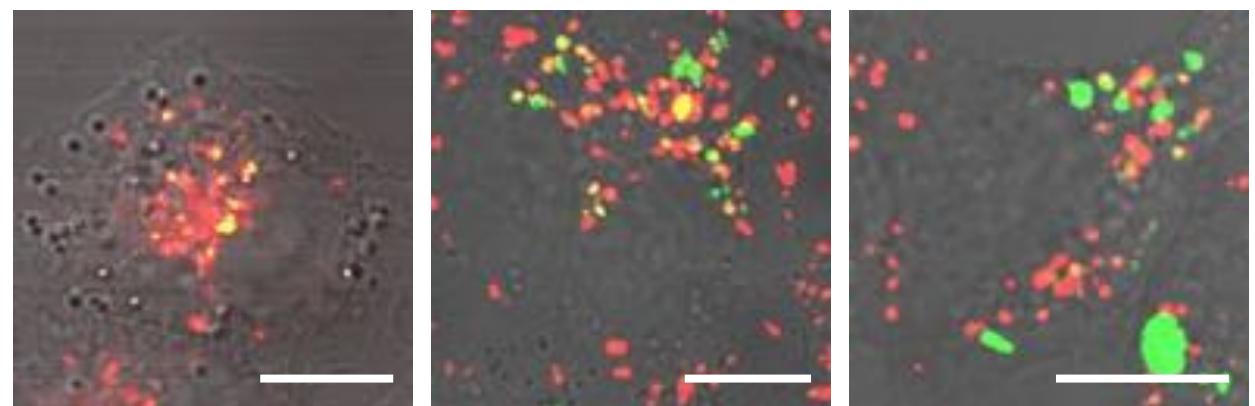
Cellular uptake and vesicle trafficking



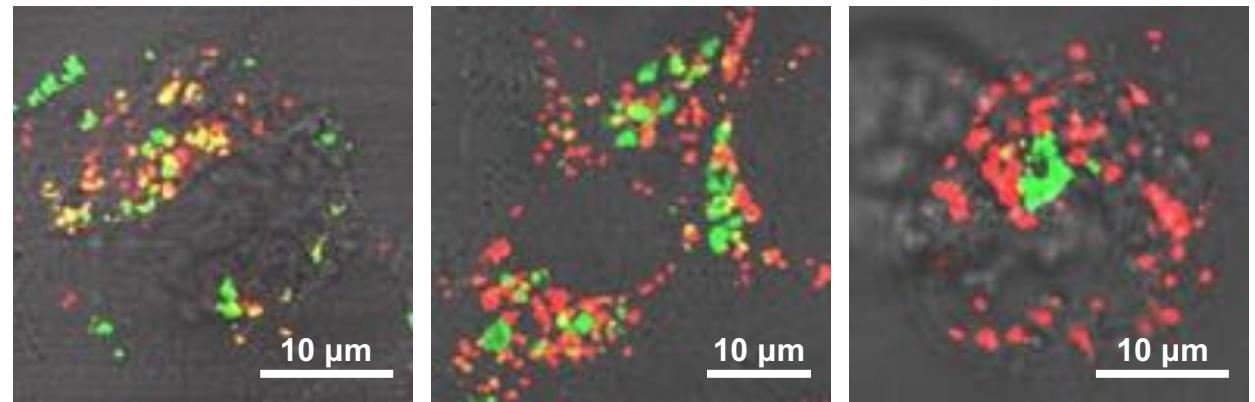
MSNPs_FITC



PEI-MSNPs_FITC



HAPEI-MSNPs_FITC

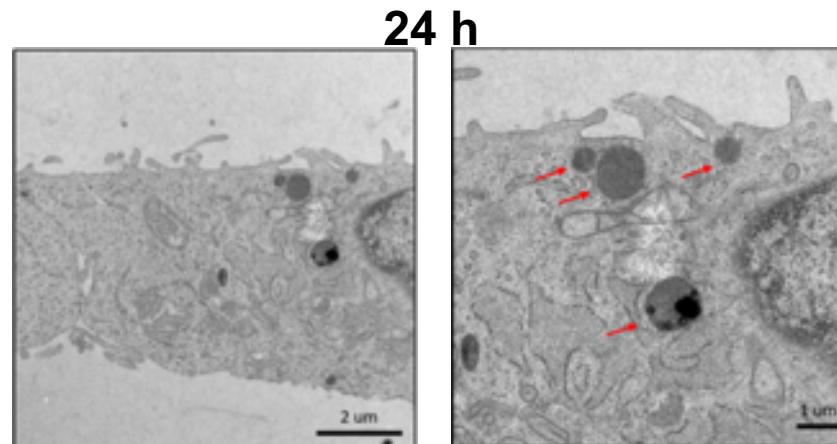


LysoTracker Red
MSNPs

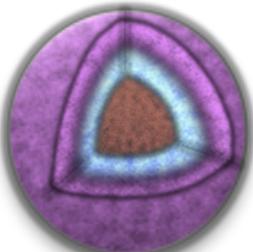
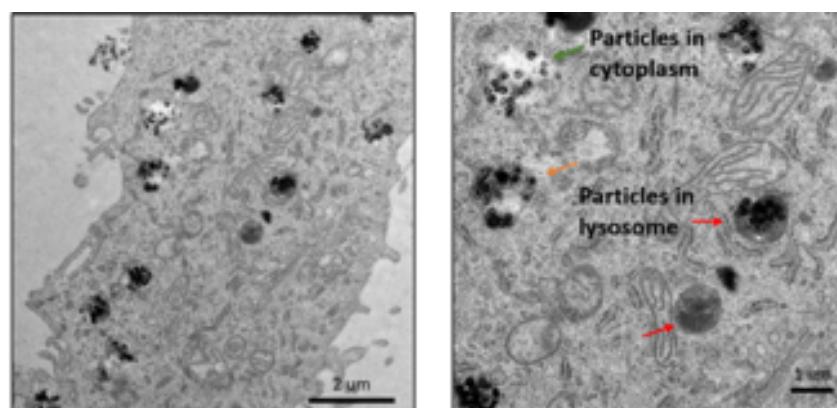
Cellular uptake and vesicle trafficking



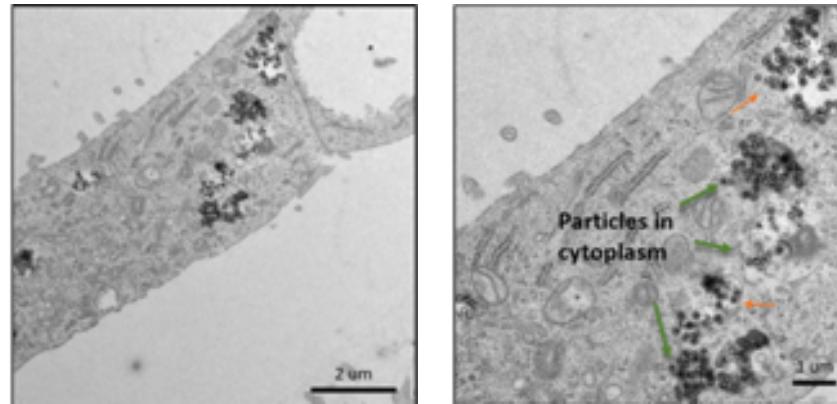
Au@MSNPs



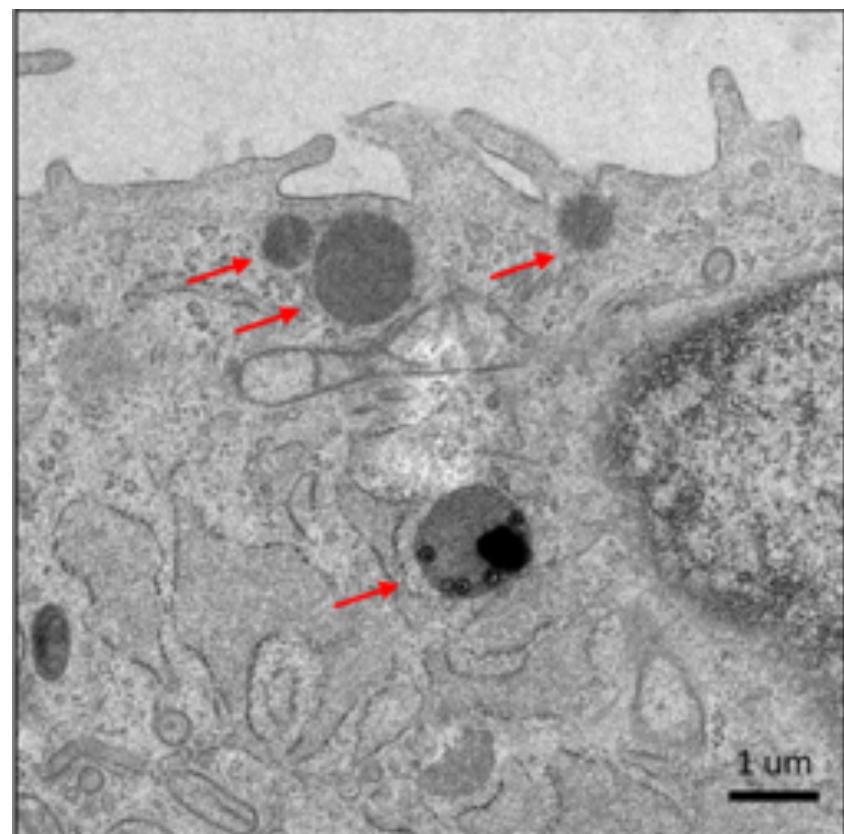
PEI-Au@MSNPs



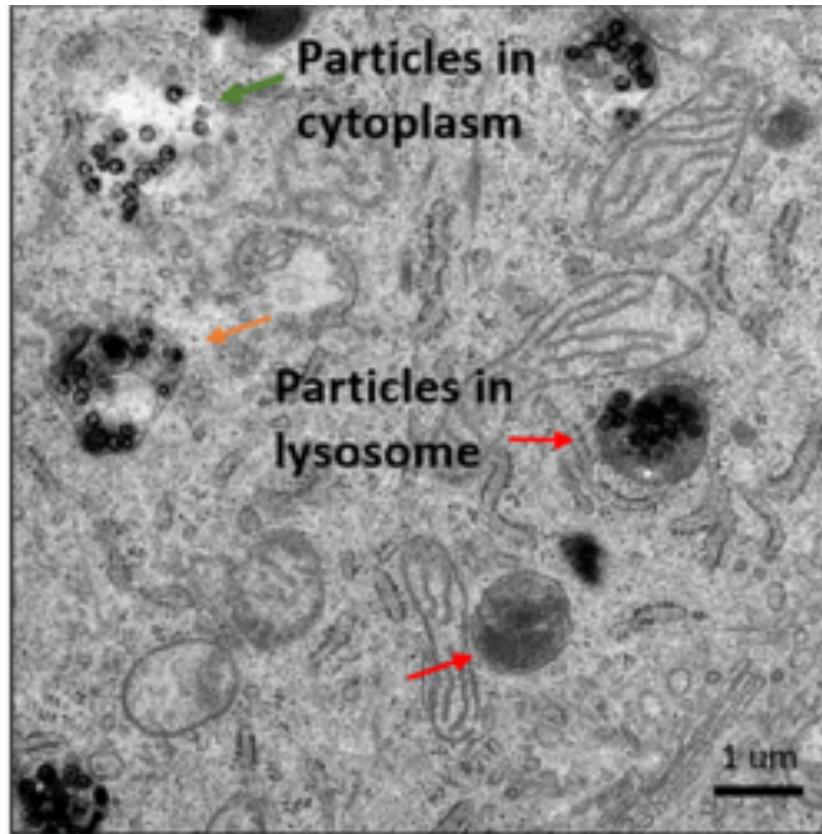
HAPEI-Au@MSNPs



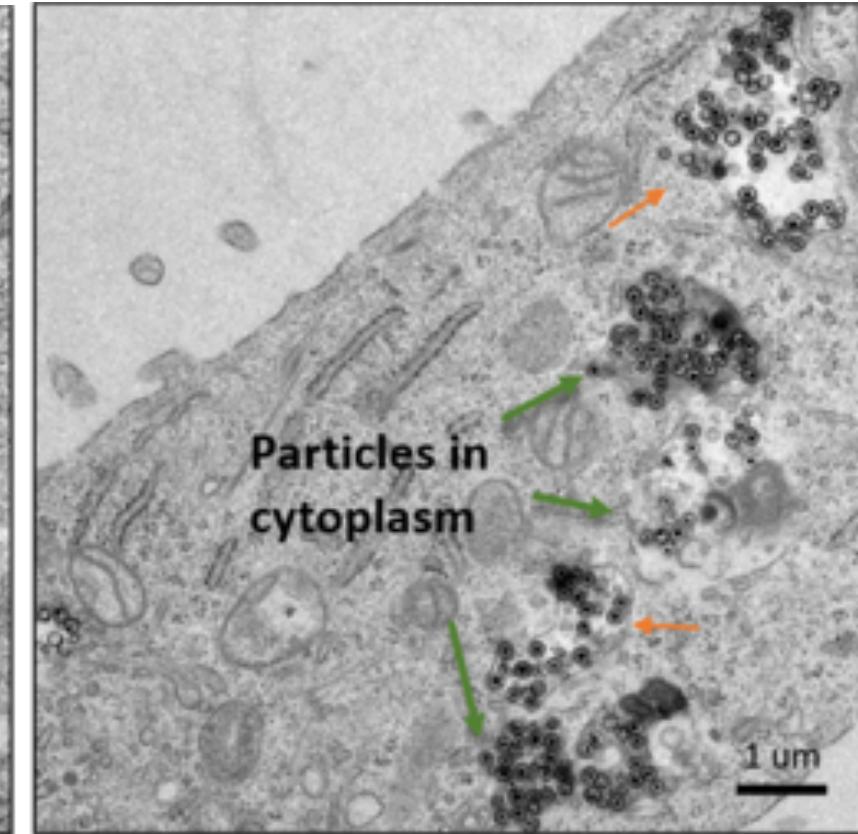
Cellular uptake and vesicle trafficking



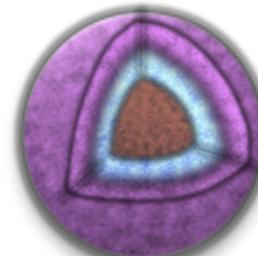
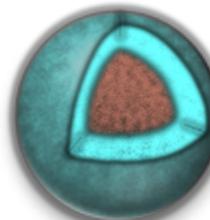
Au@MSNPs



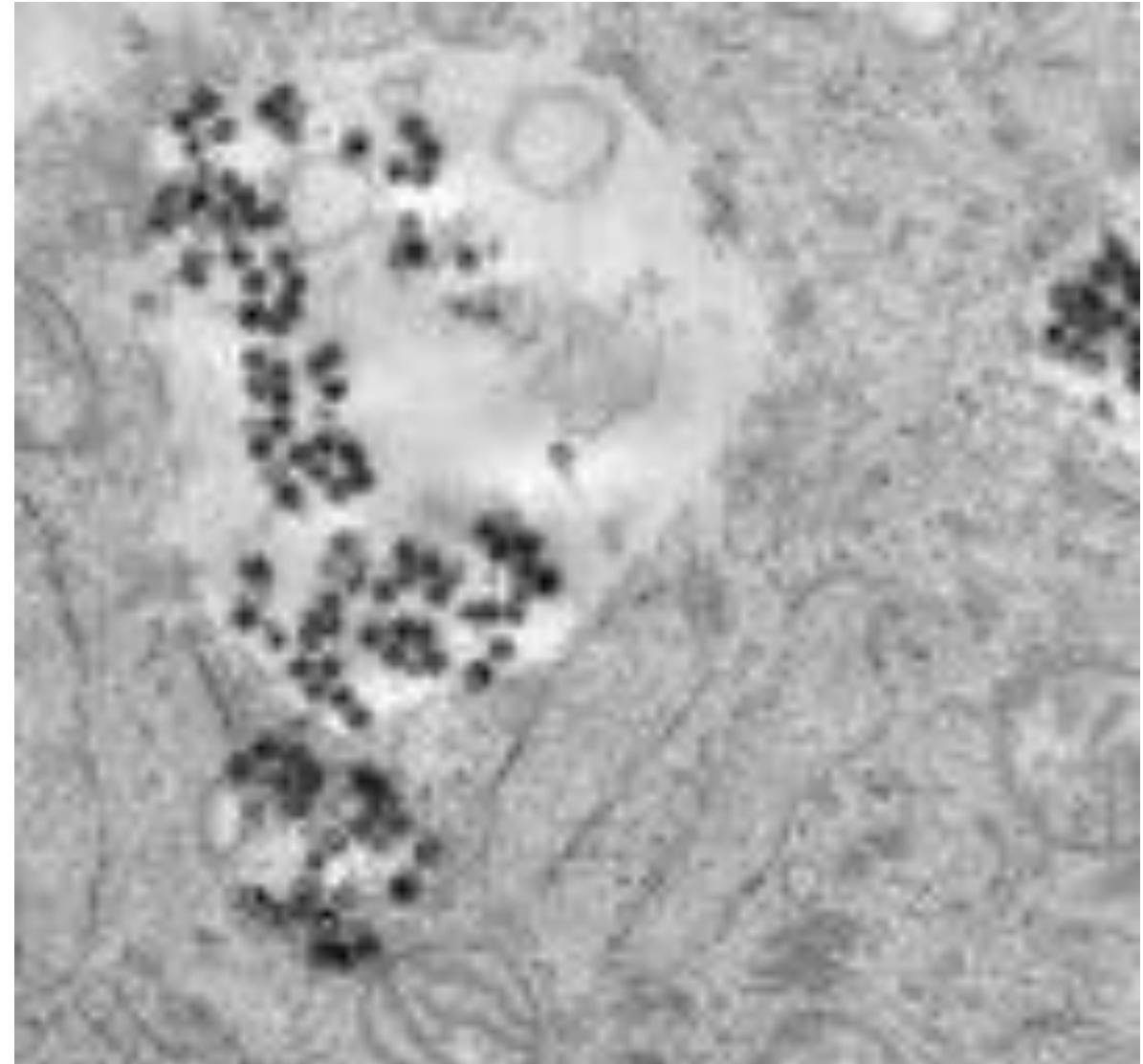
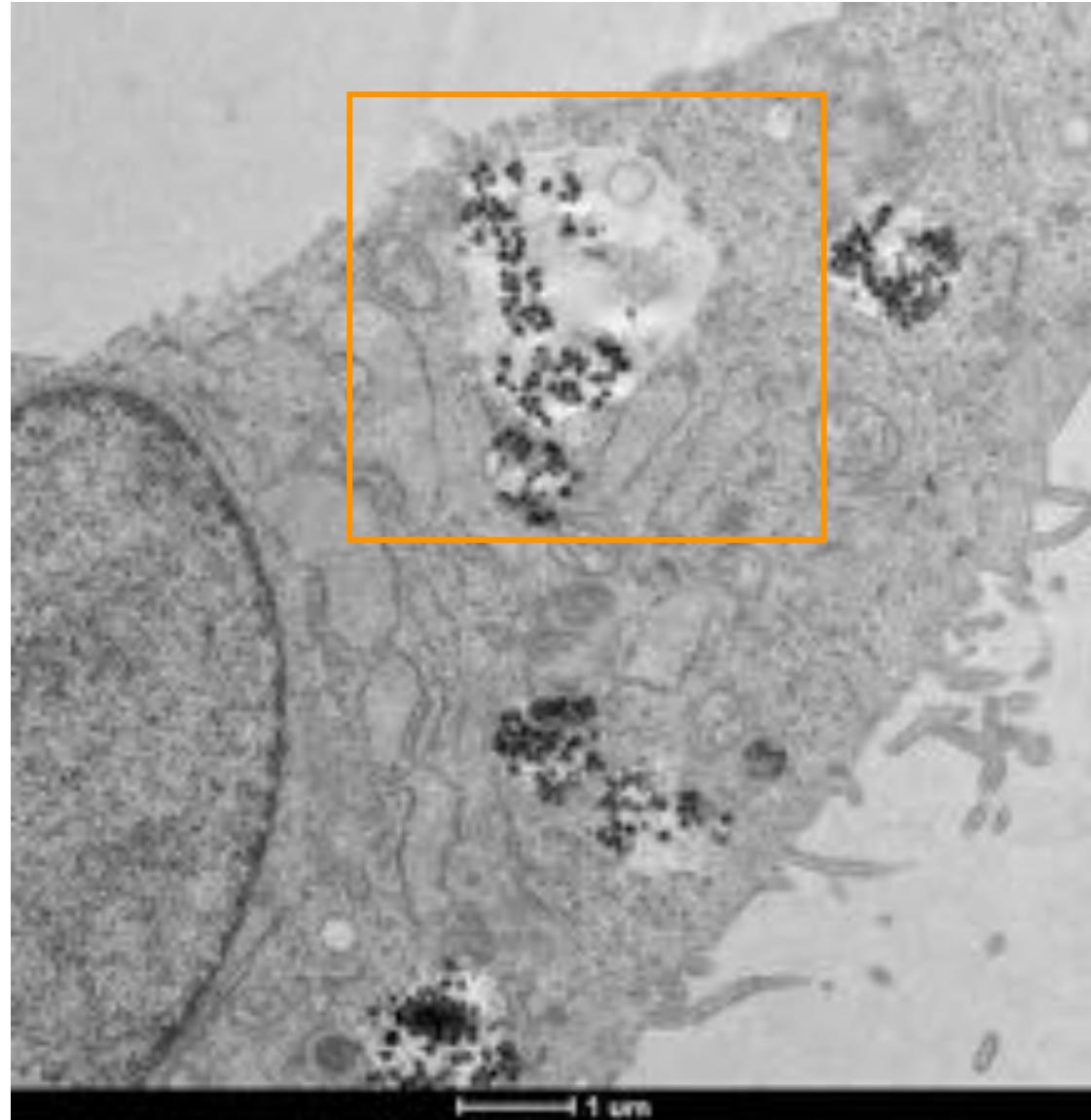
PEI-Au@MSNPs



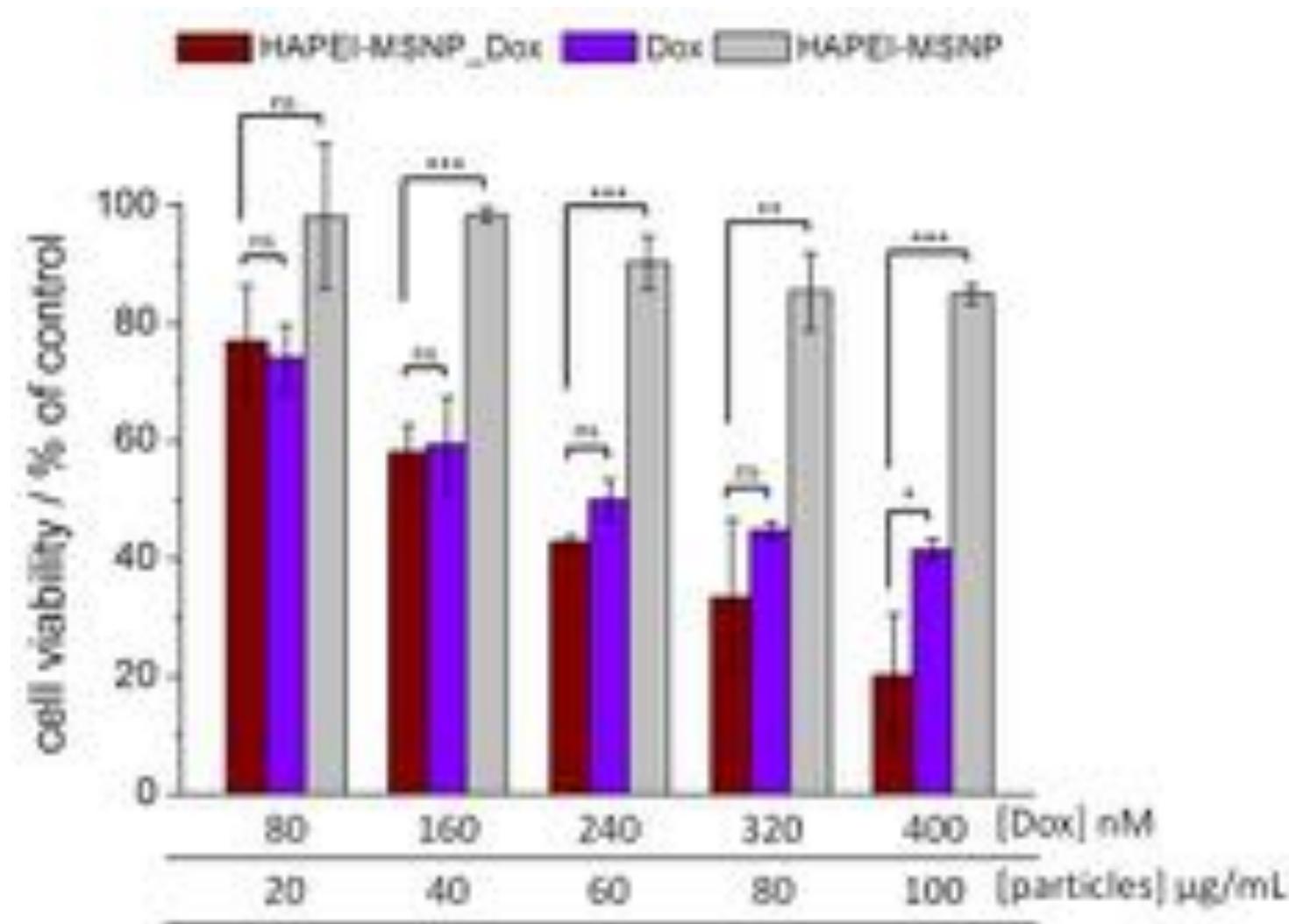
HAPEI-Au@MSNPs

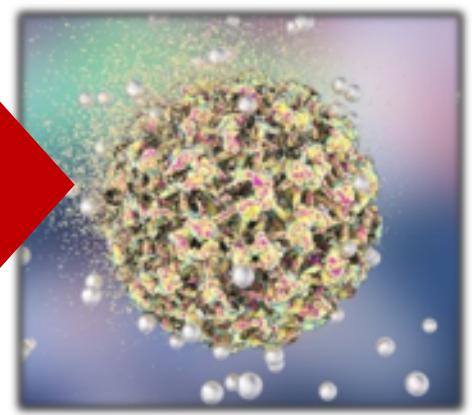


Endosomal rupture?!?

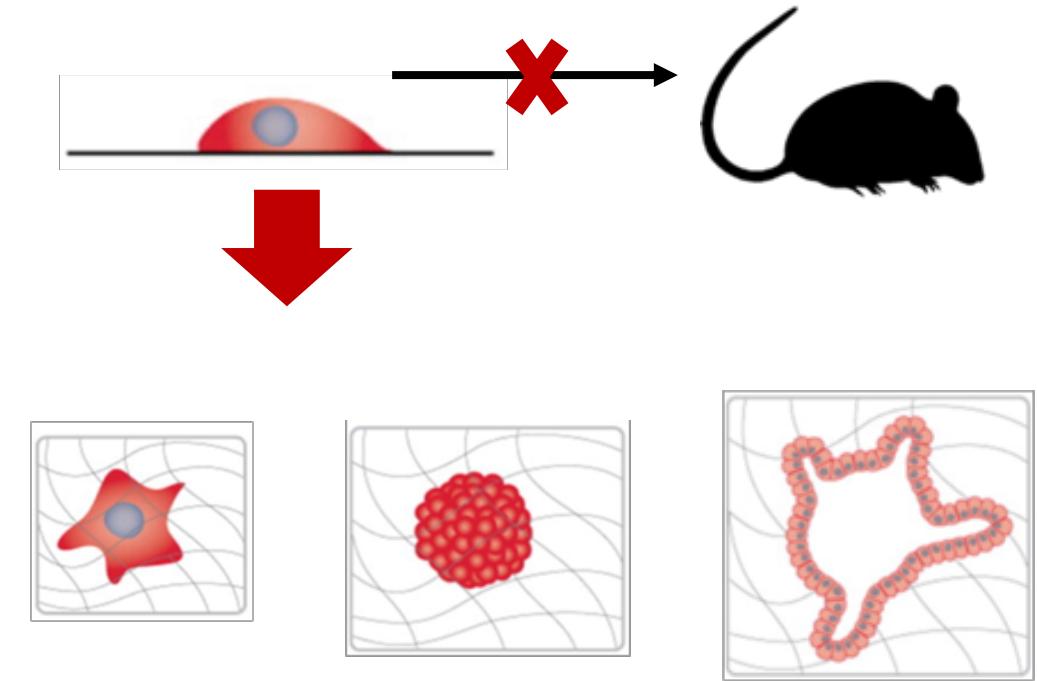


Anticancer efficiency of Dox-loaded particles



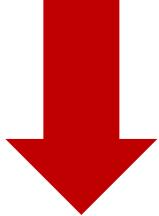


The (nano)medicine



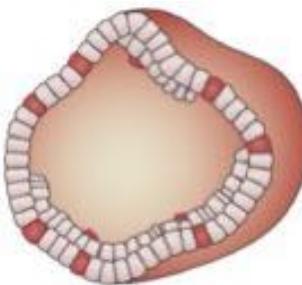
The model

What is the
intercellular route of
the nanoparticles?



**Use of multi-cellular organoids
as a tumor mimicking system**

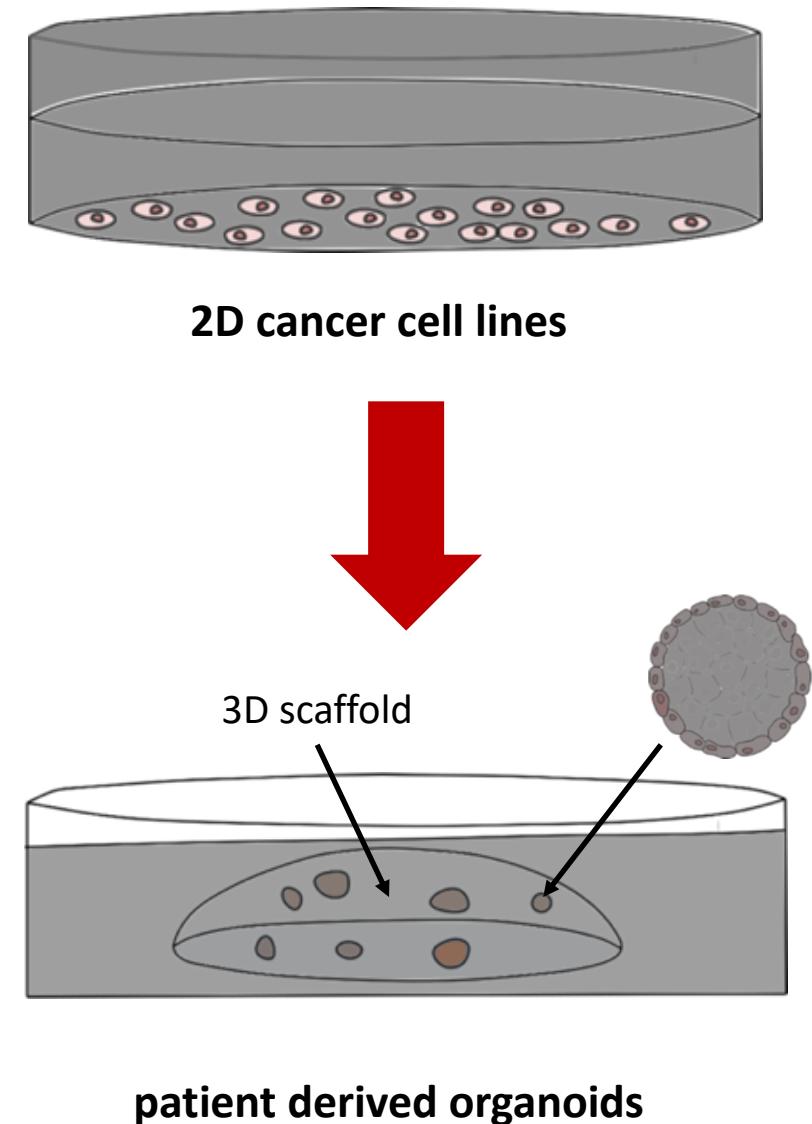
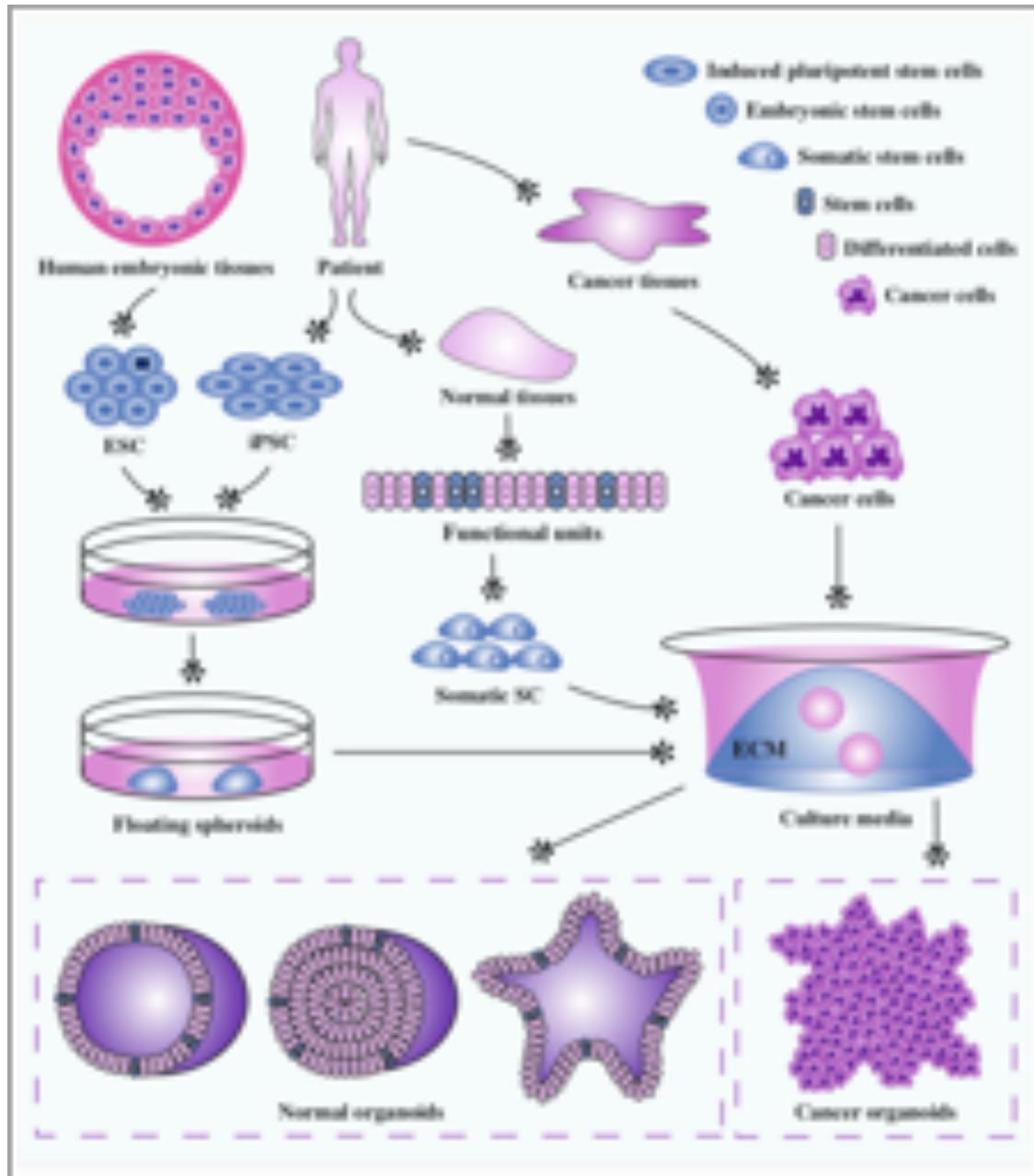
Diffusion



Transcytosis
or/and para
cellular
transport?

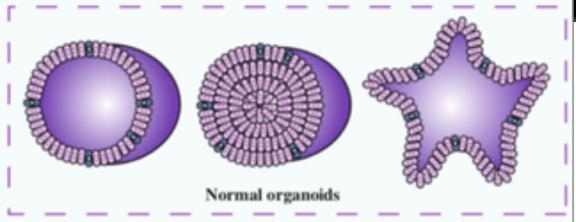
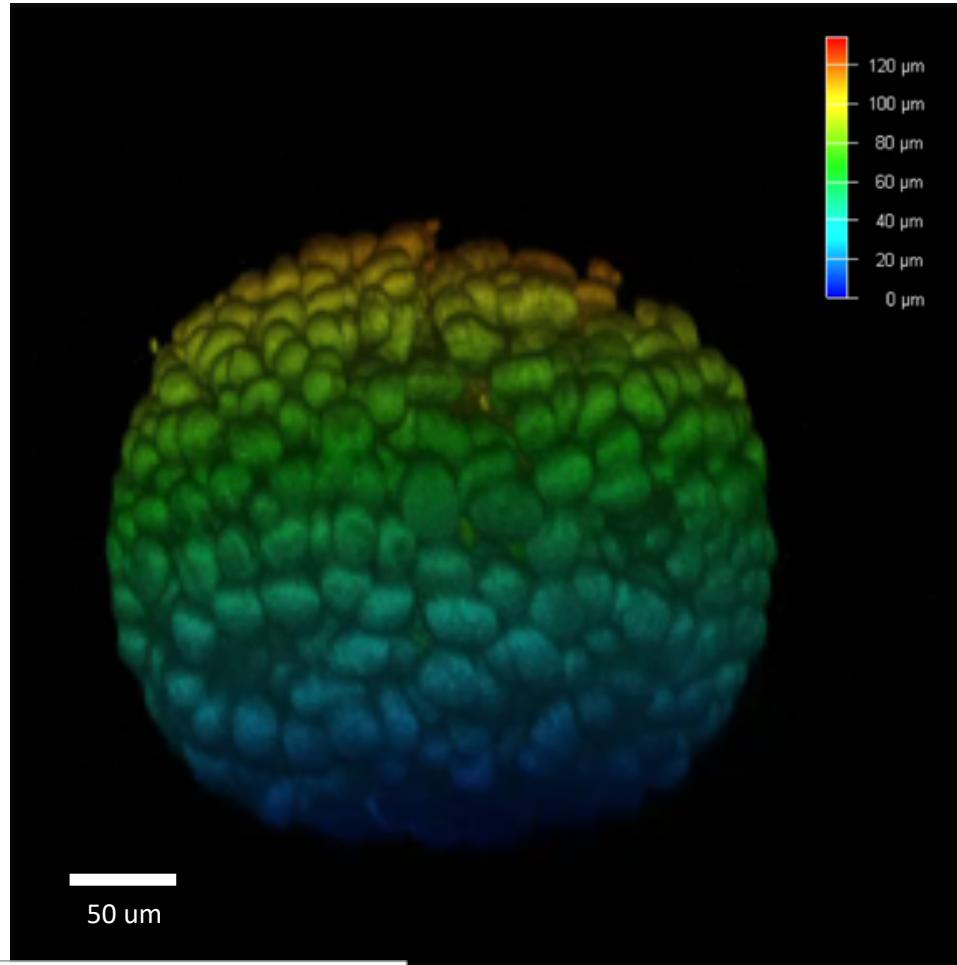
Permeability

Organoids as realistic *in vitro* cancer models

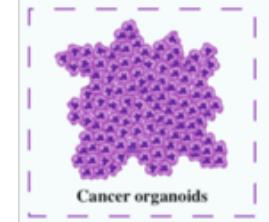
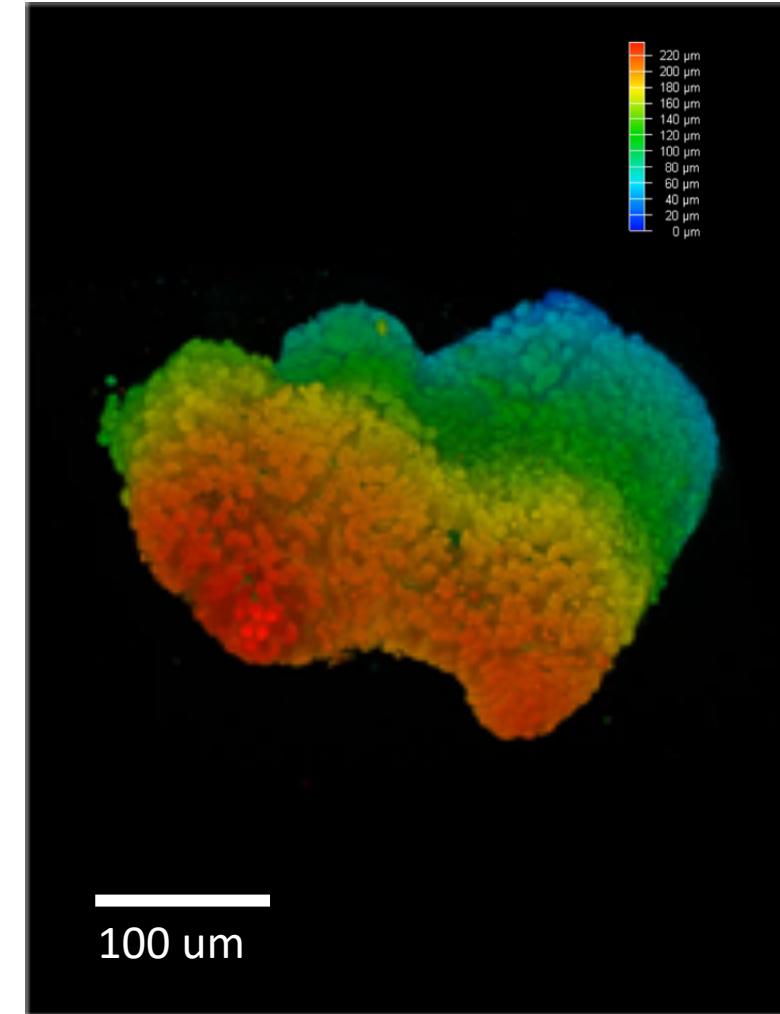


Organoids as realistic *in vitro* cancer models

Healthy endometrial organoid

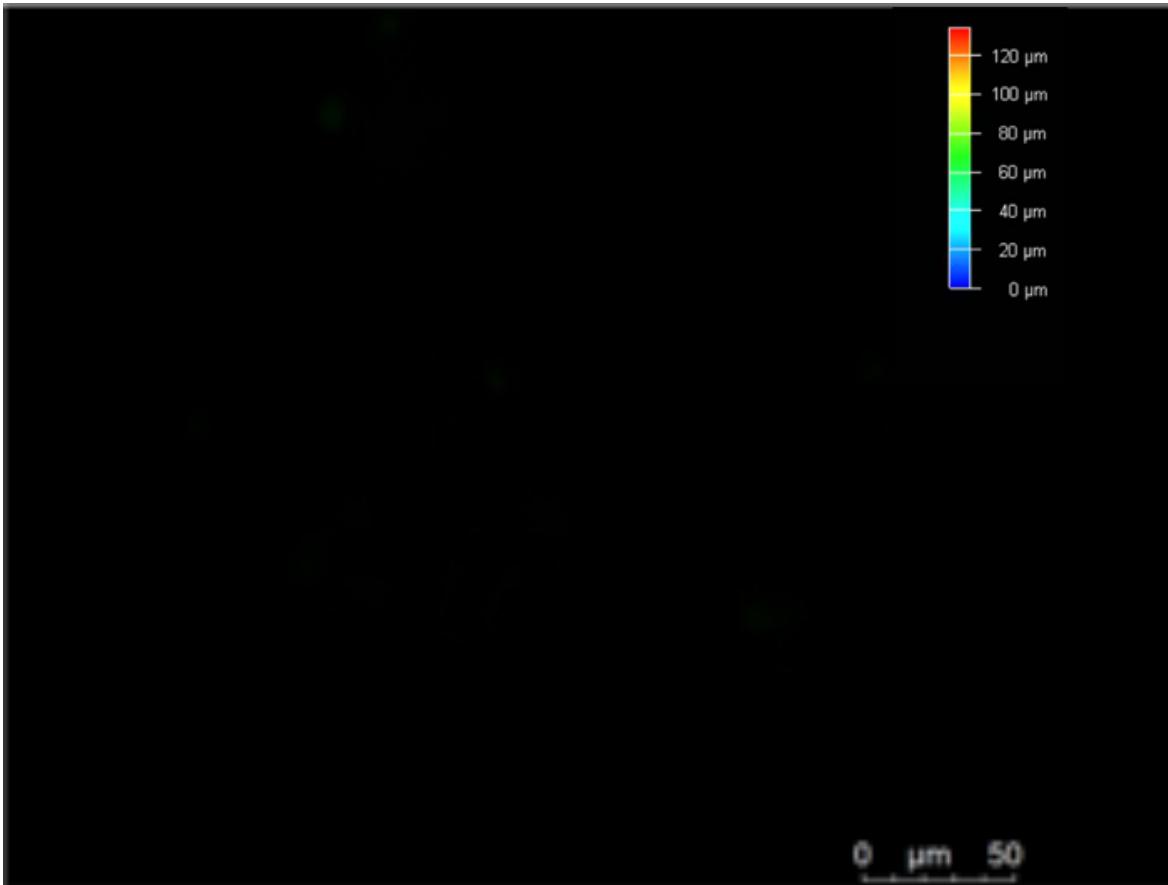


endometrial cancer organoid

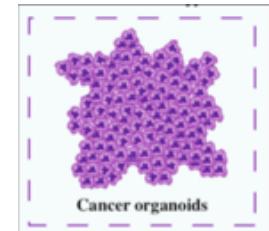
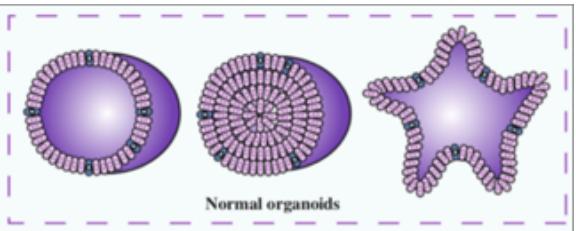
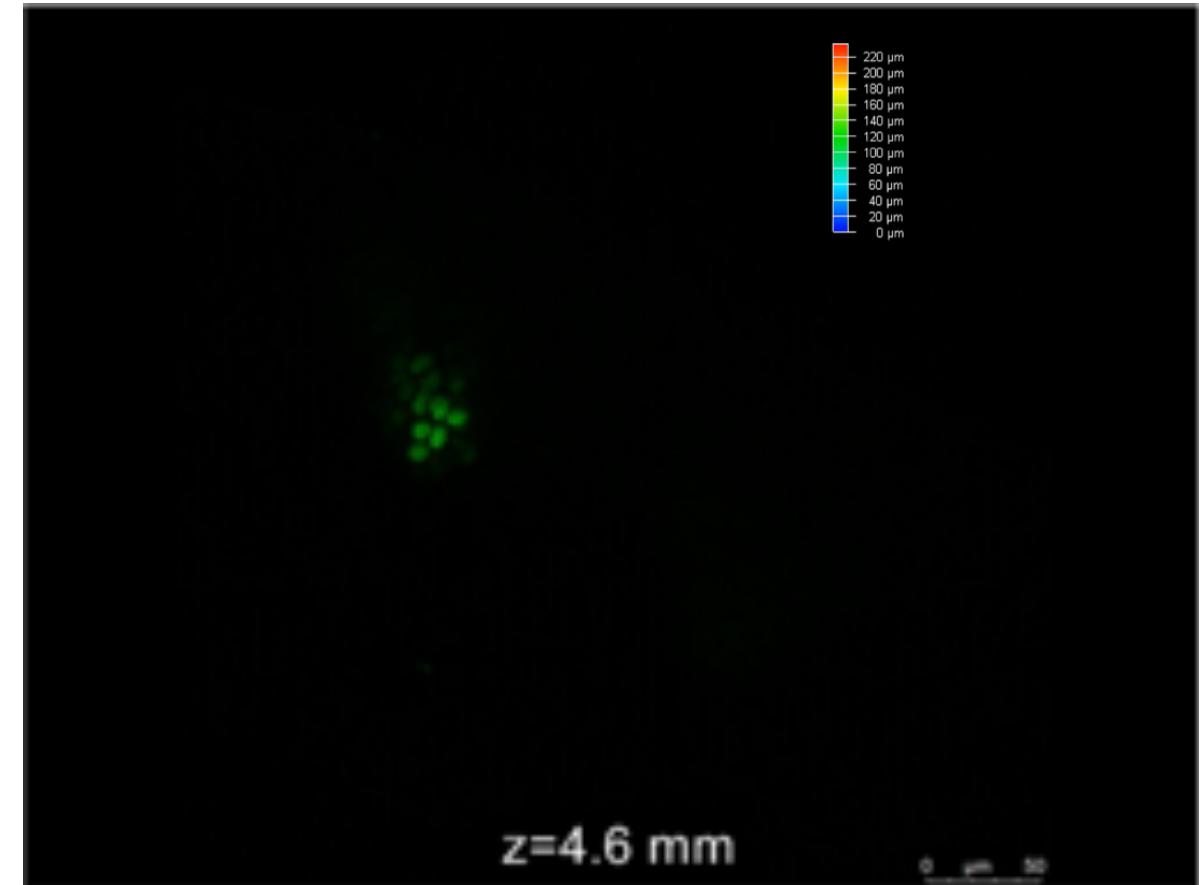


Organoids as realistic *in vitro* cancer models

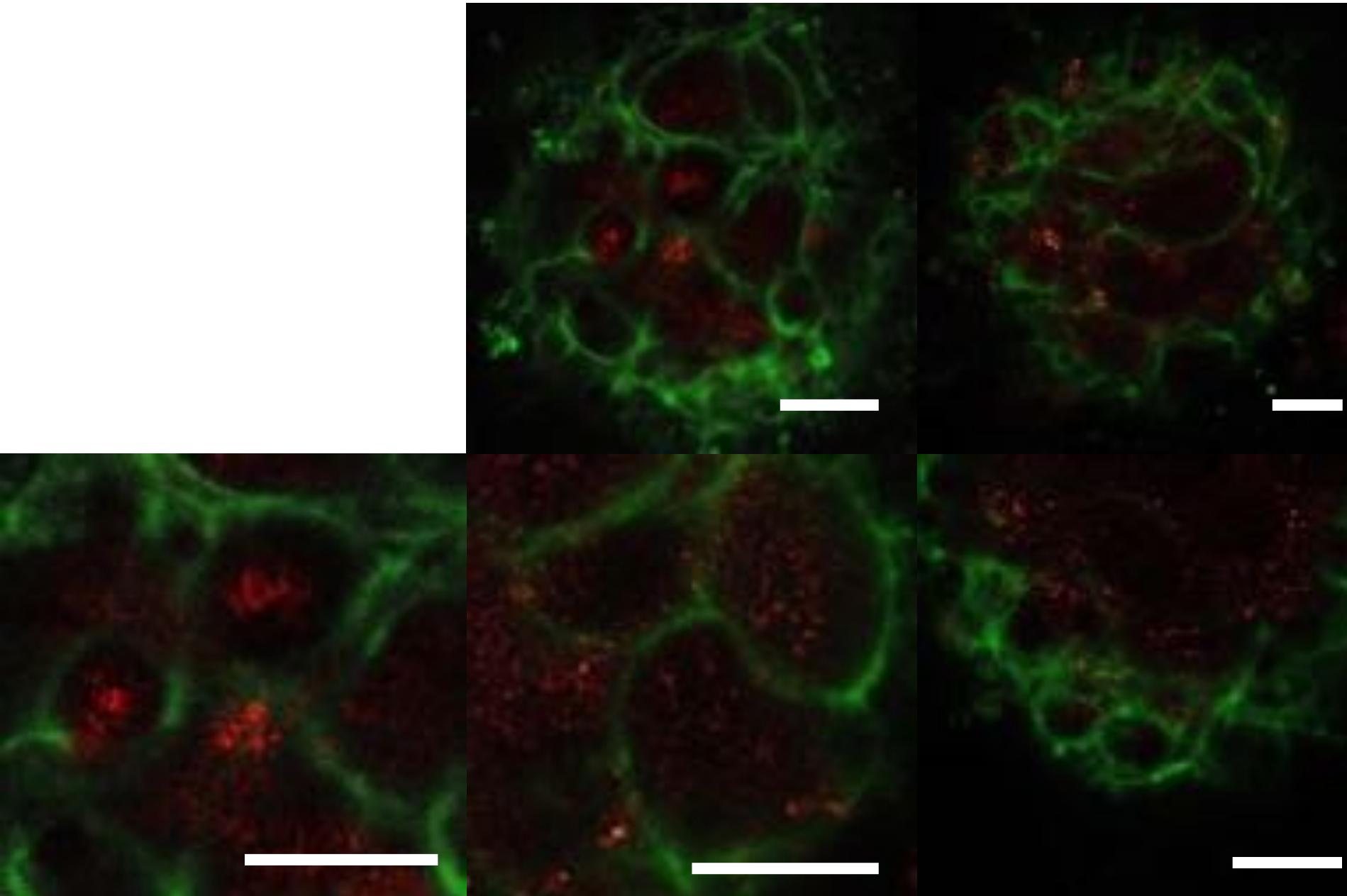
Healthy endometrial organoid



endometrial cancer organoid



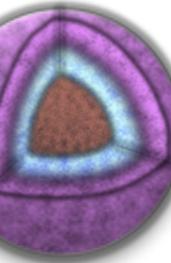
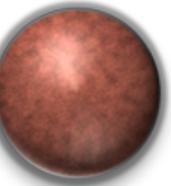
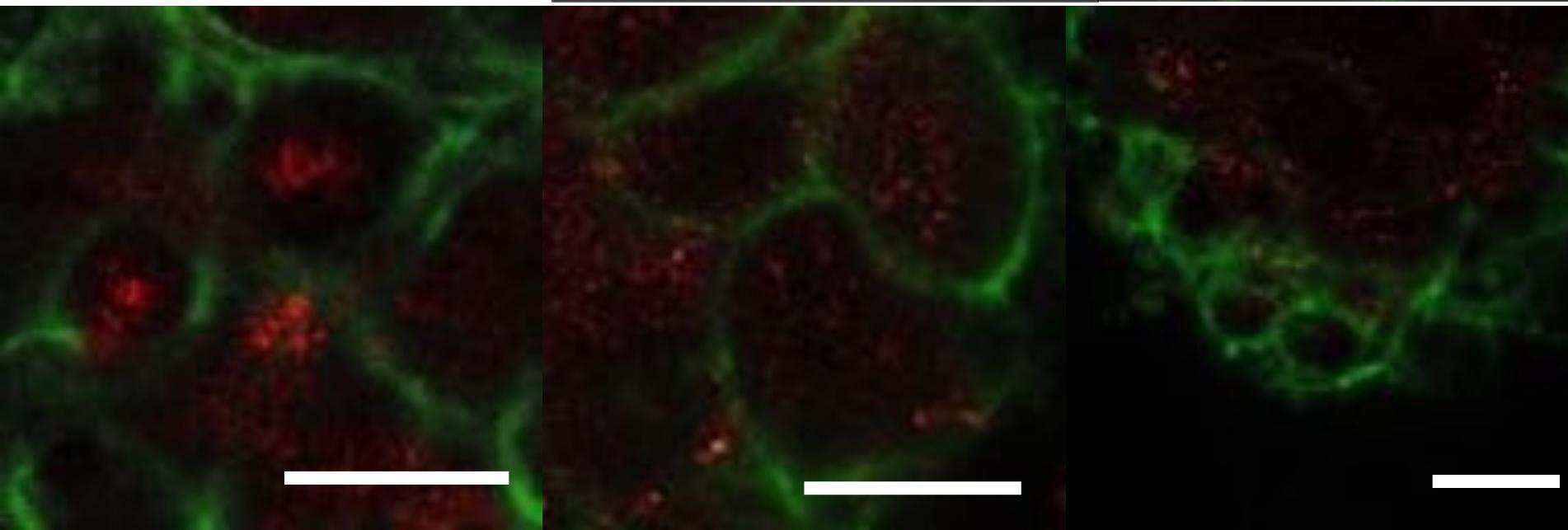
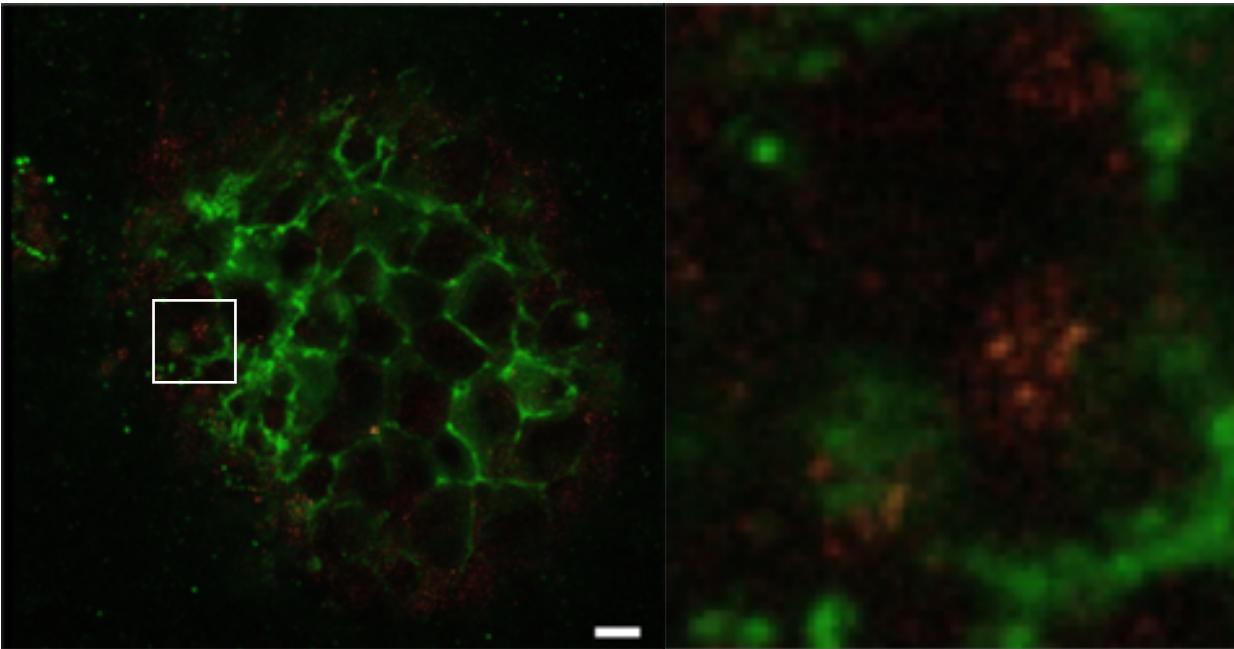
Particle uptake in patient-derived organoids



HAPEI MSNP RhoB
CD44 (secondary alexa 488)

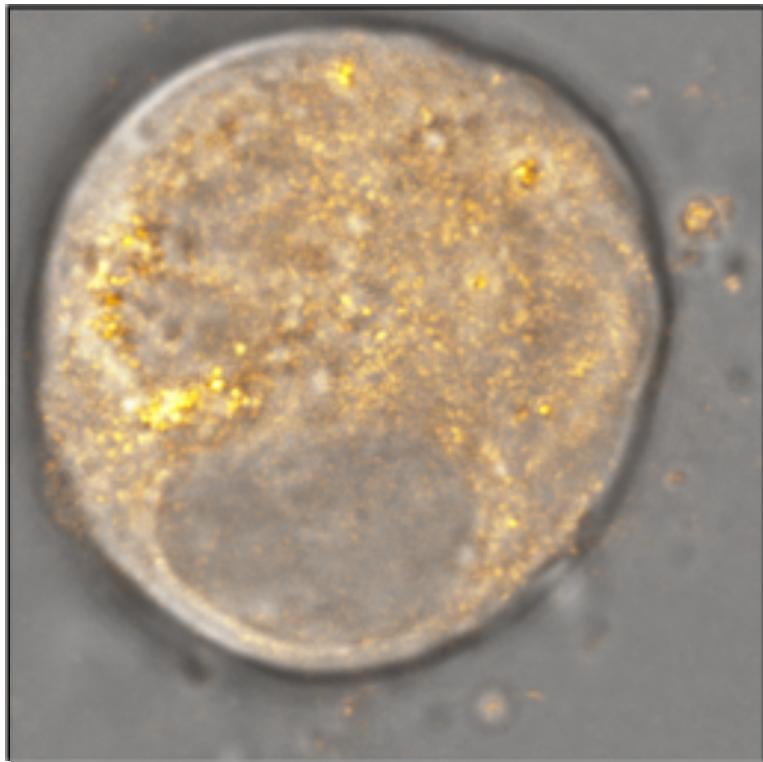
Scale = 10 um

Particle uptake in patient-derived organoids

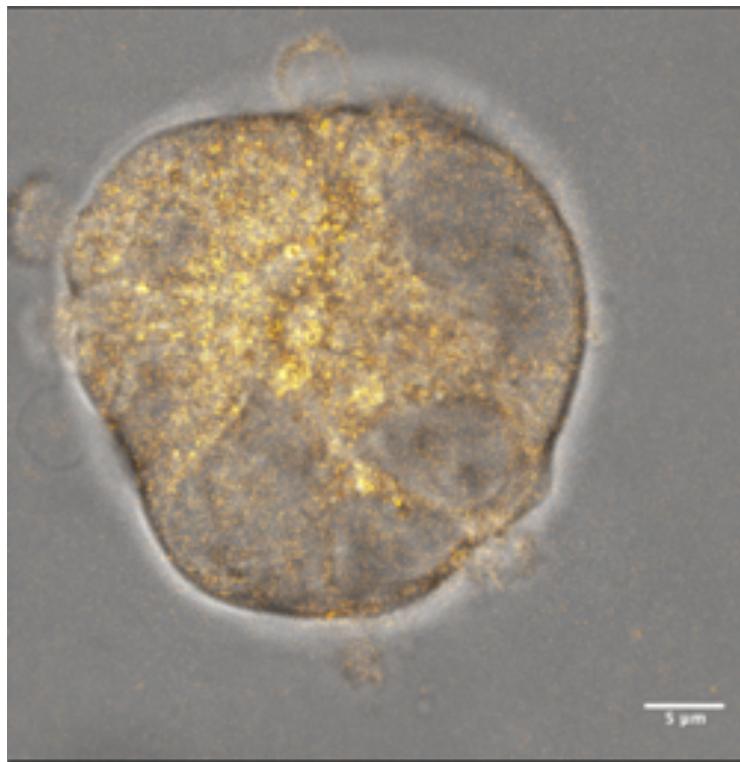


Scale = 10 um

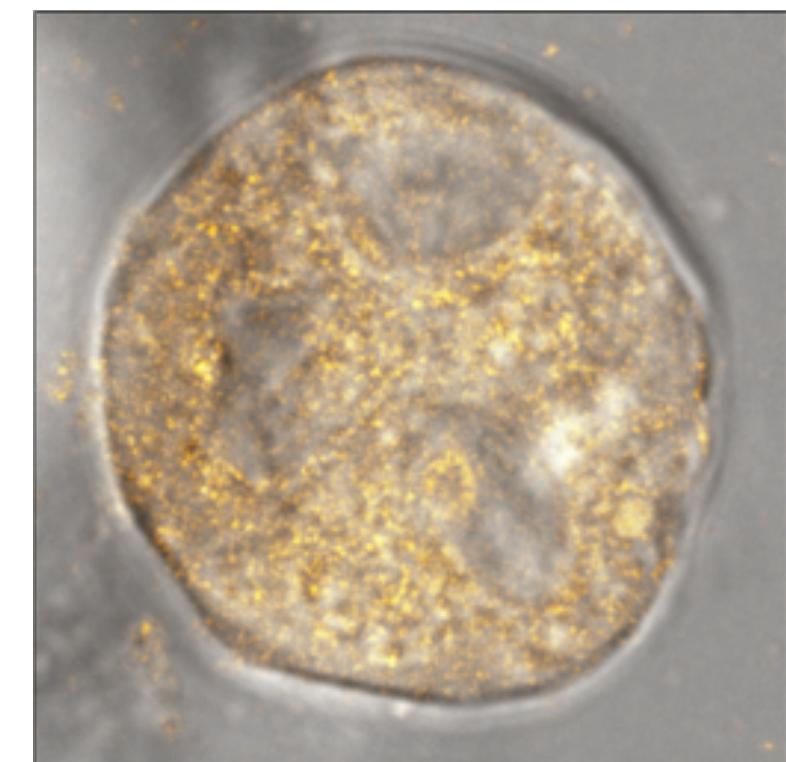
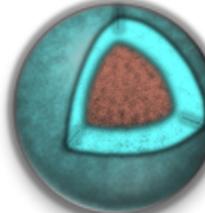
Particle uptake in patient-derived single cells



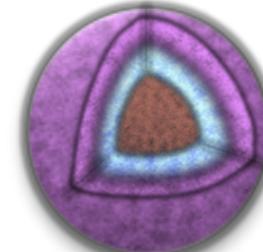
MSNPs



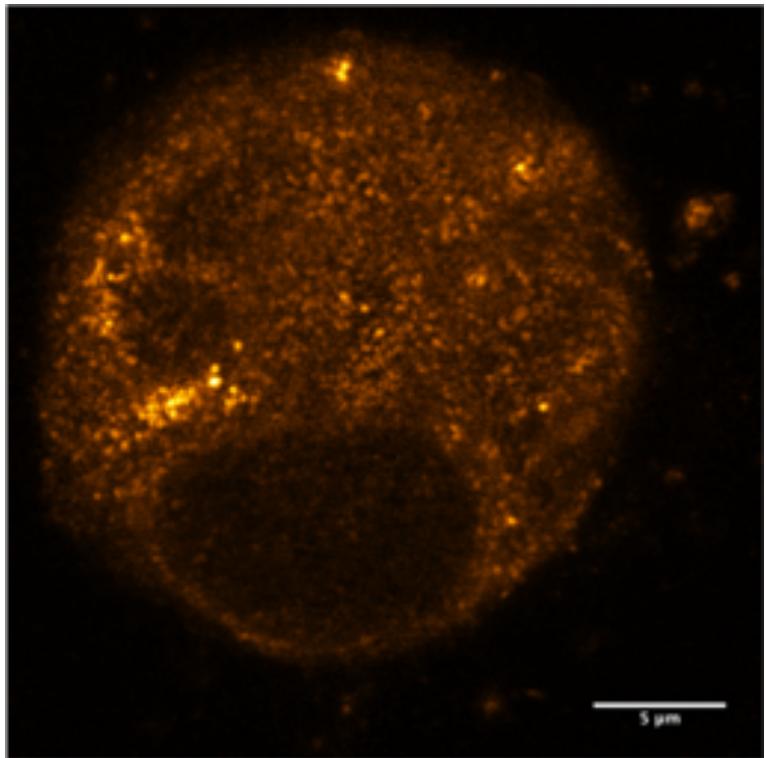
PEI-MSNPs



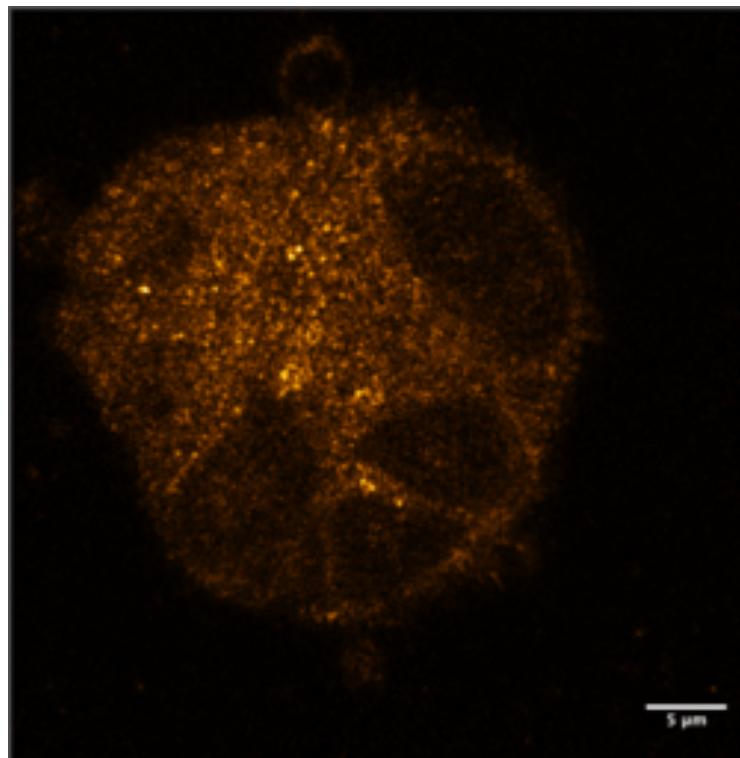
HAPEI-MSNPs



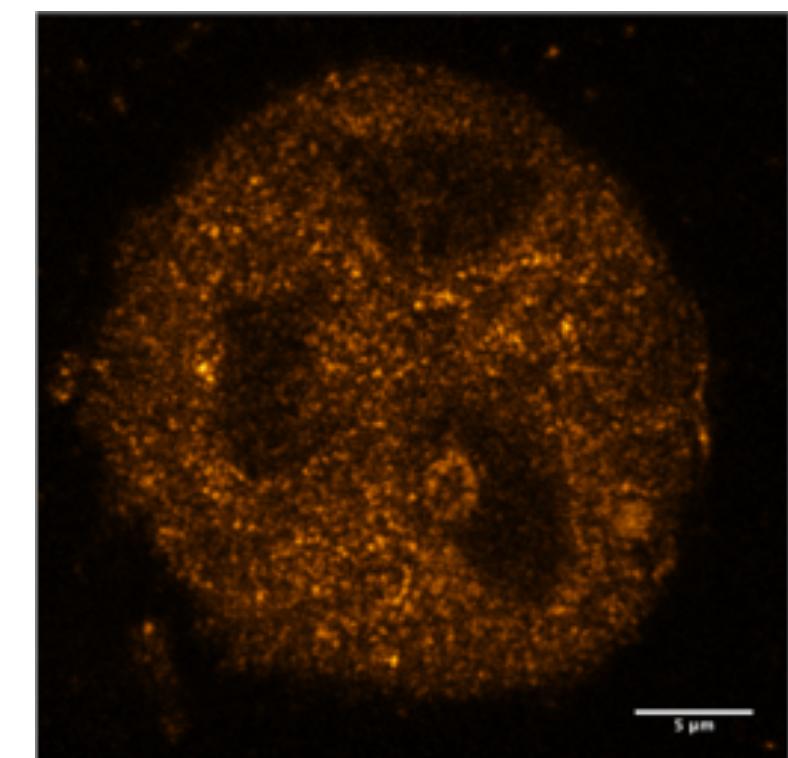
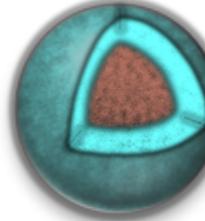
Particle uptake in patient-derived single cells



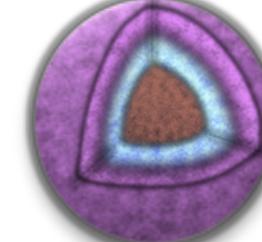
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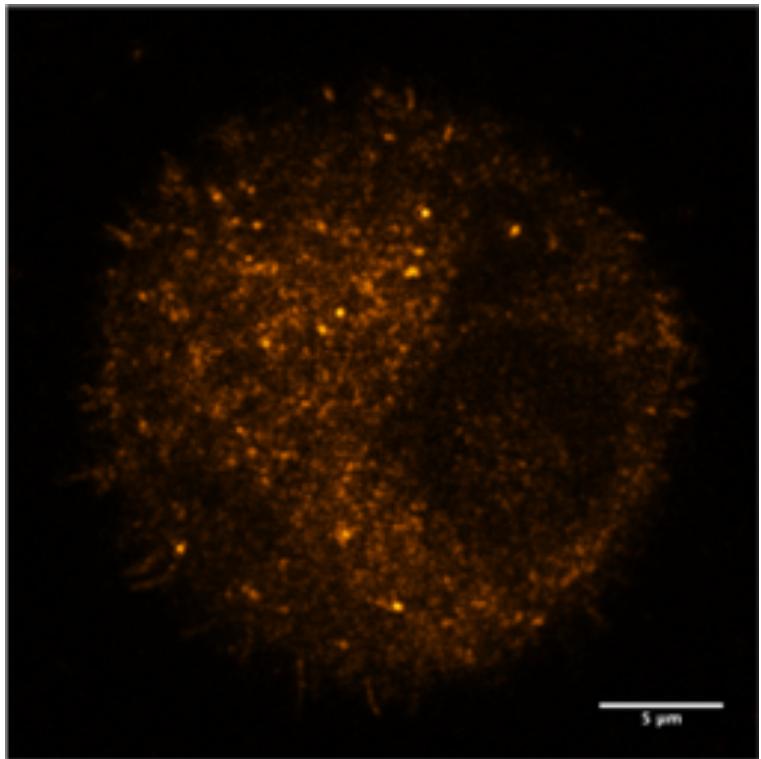
PEI-MSNPs



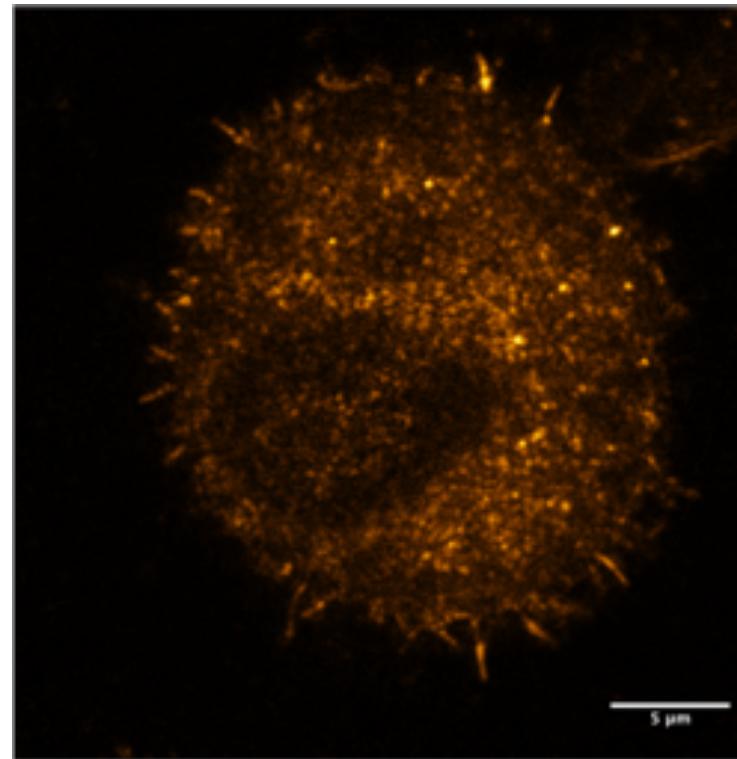
HAPEI-MSNPs



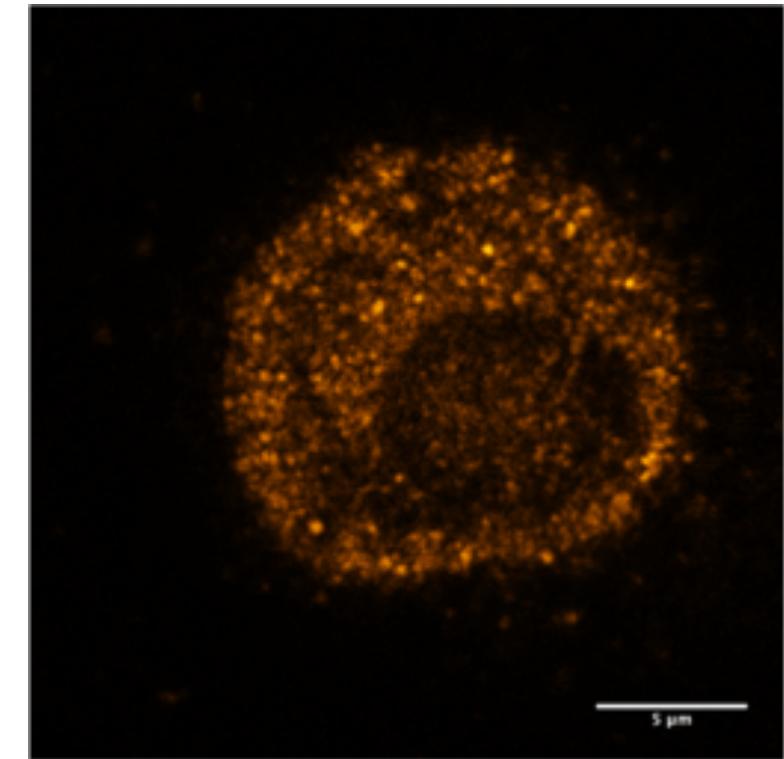
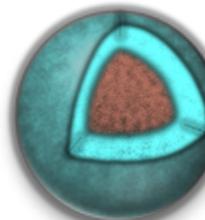
Particle uptake in A549



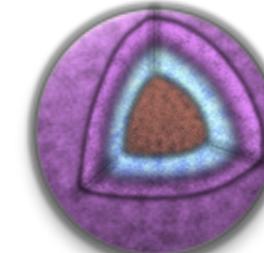
MSNPs



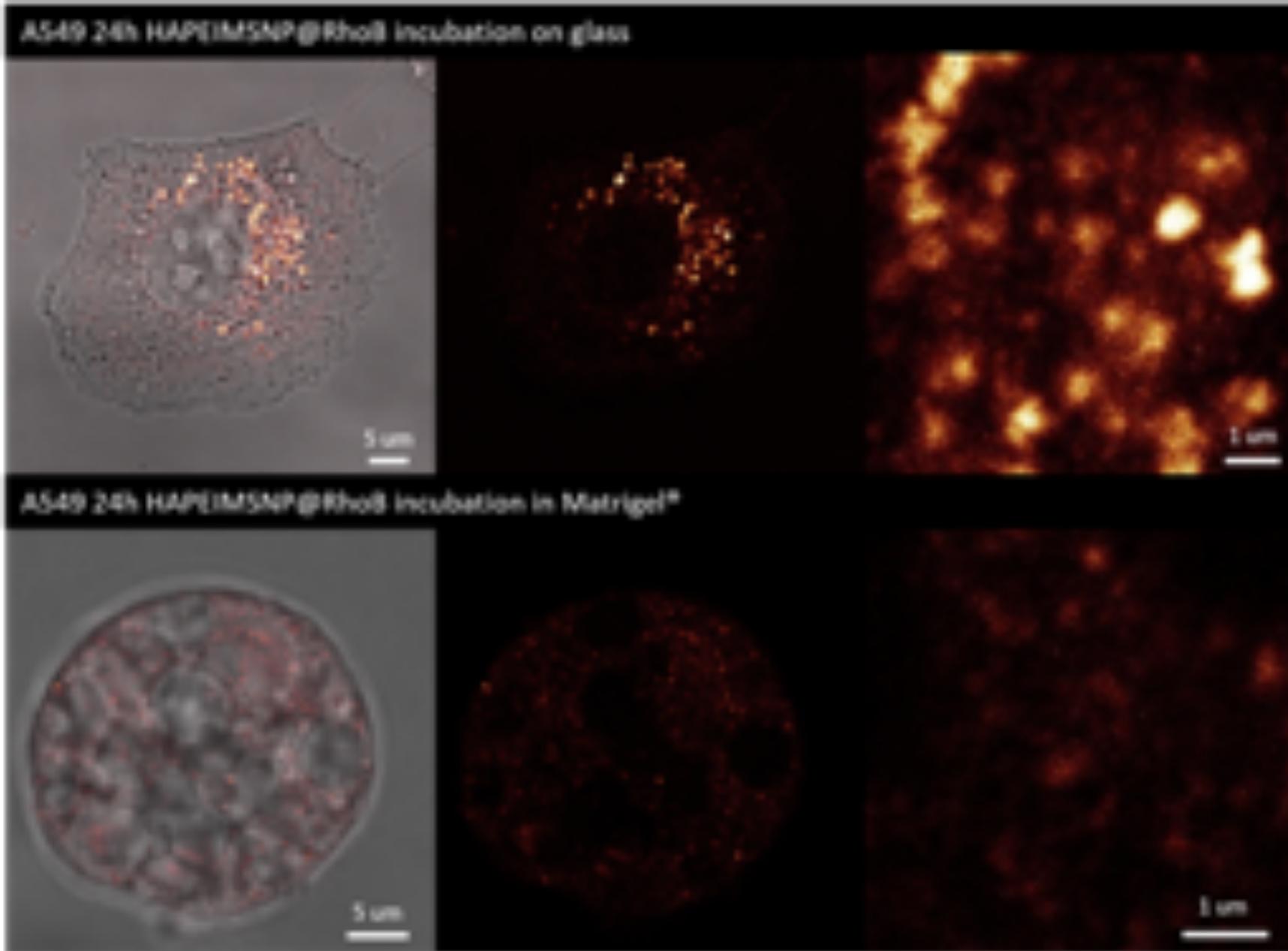
PEI-MSNPs



HAPEI-MSNPs



Particle uptake in A549



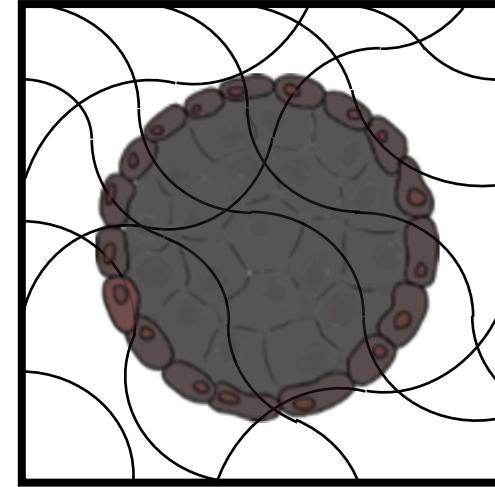
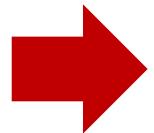
HAPEI-MSNPs





2D on glass (A549 cells)

Specific uptake of HA-coated NP



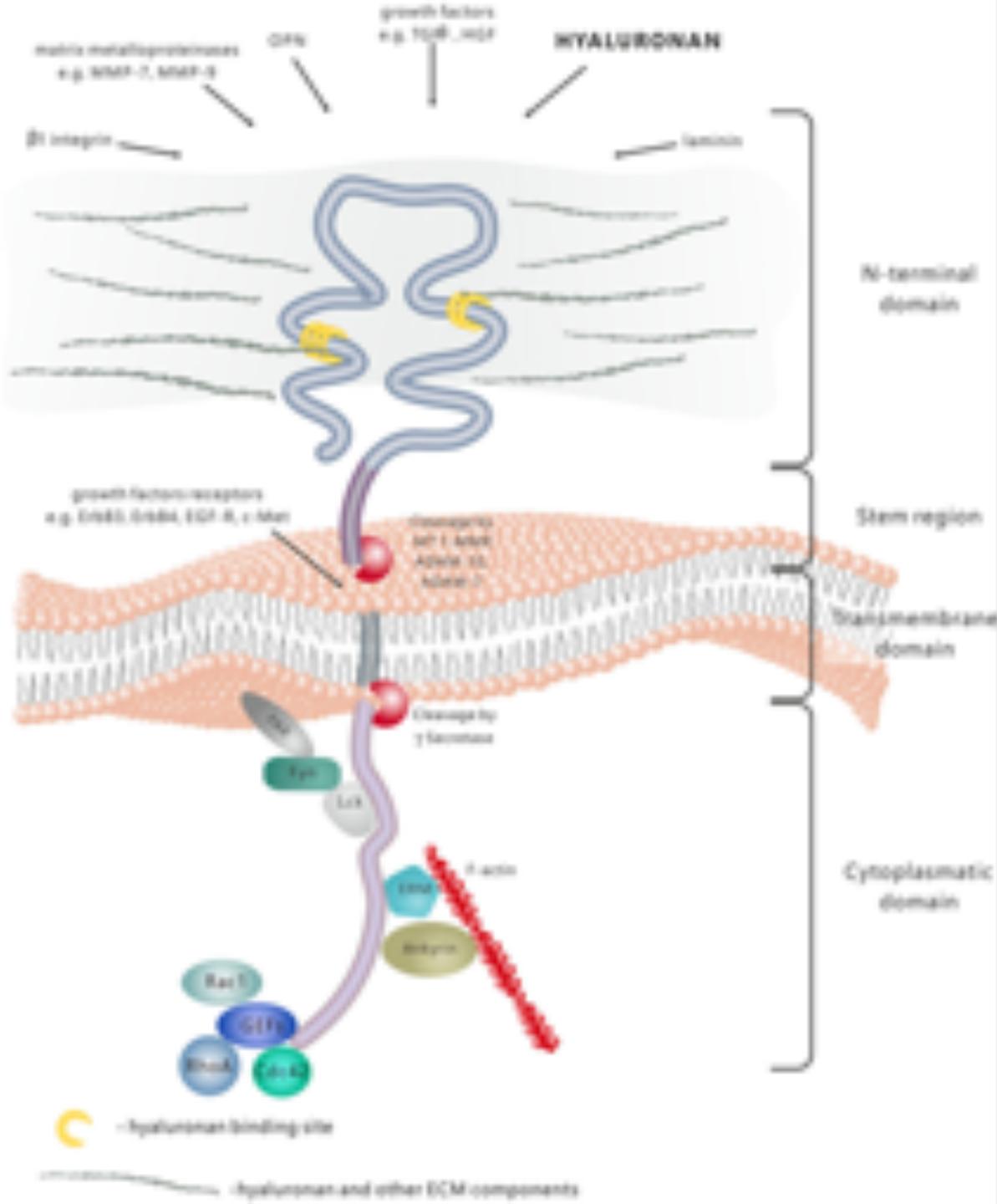
3D in Matrigel (organoids and A549)

Uptake of both HA-coated and non-coated particles

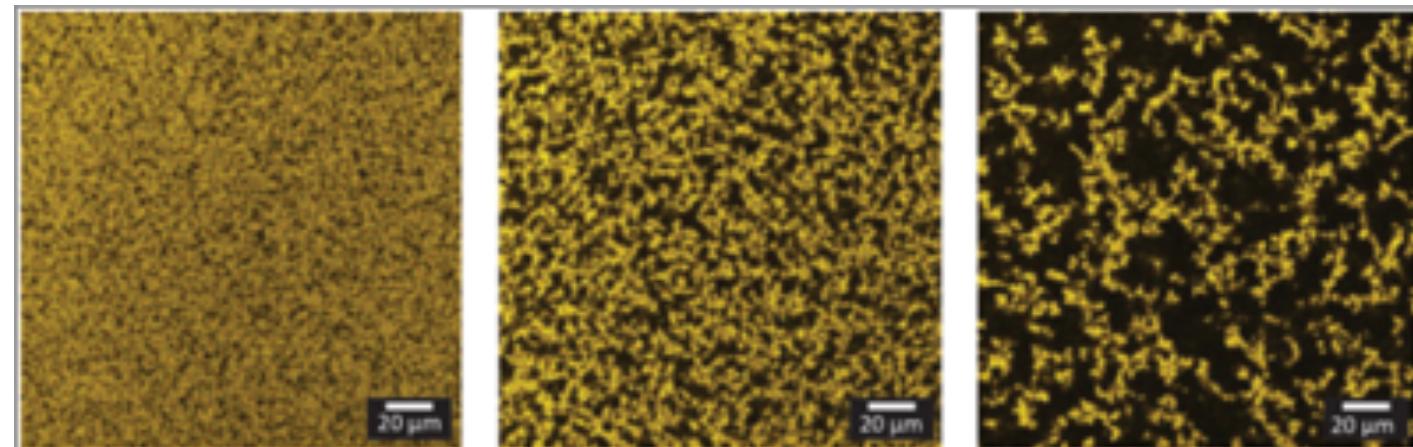
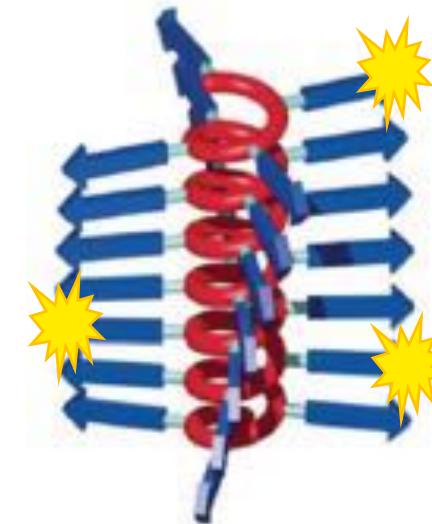
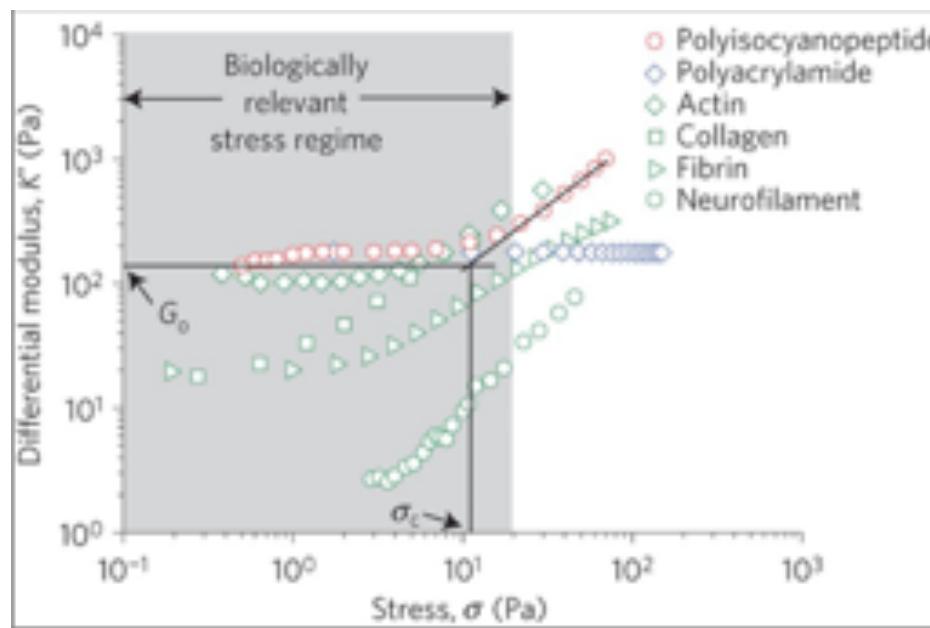
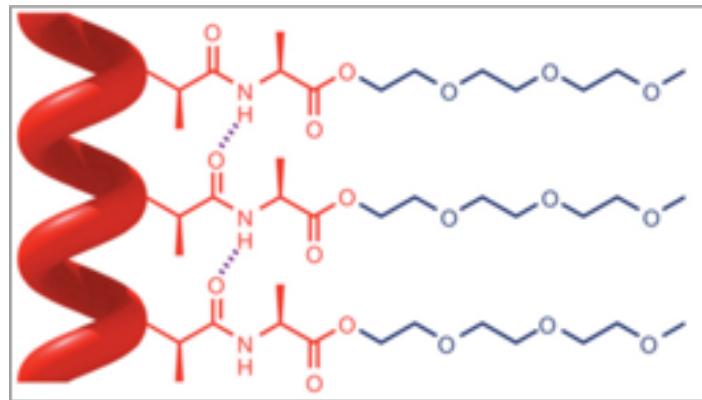


Role of CD44 receptor

The N-terminal extracellular domain can bind various ligands, including hyaluronan (HA), extracellular matrix (ECM) glycoproteins and proteoglycans, growth factors, cytokines and matrix metalloproteinases.



Minimalist matrix: Polyisocyanopeptides (PIC)



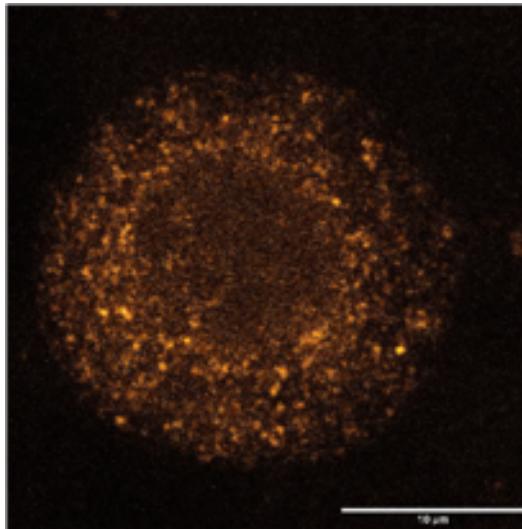
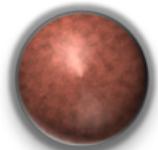
Nature, 493, 651-655 (2014), doi: 10.1038/nature11839

Nature Communications, 5, 5808 (2014), doi: 10.1038/ncomms6808

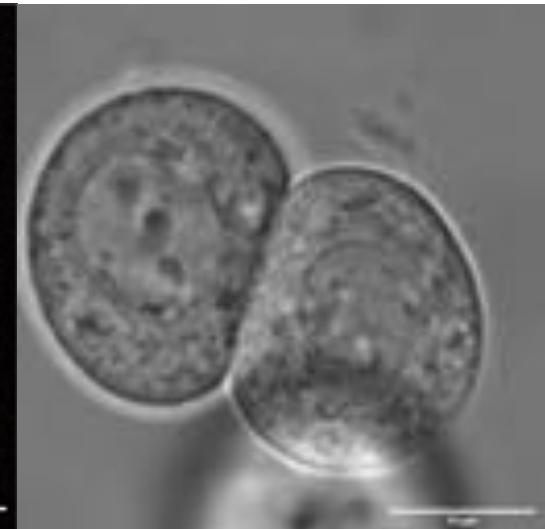
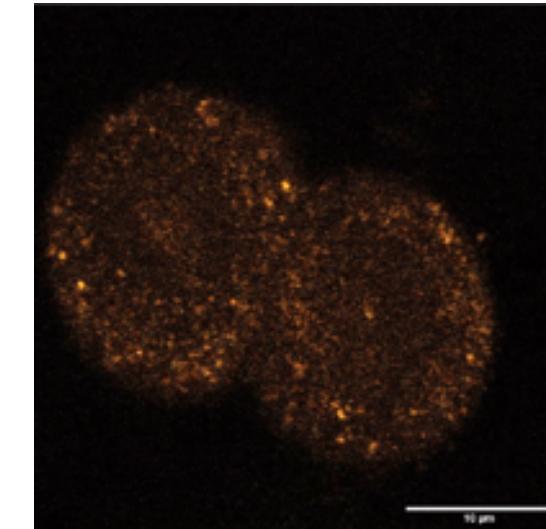
Particle uptake in 3D matrix

Matrigel

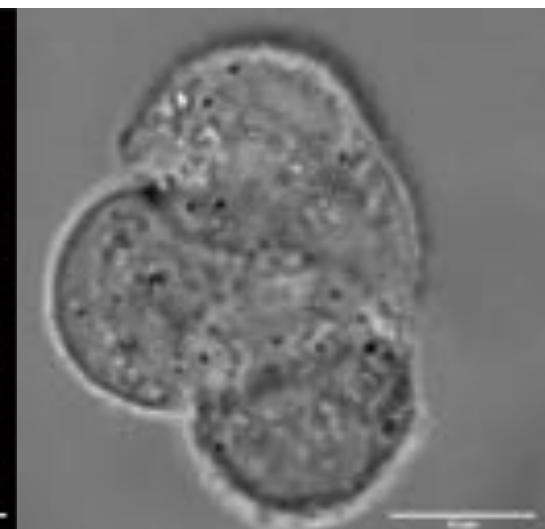
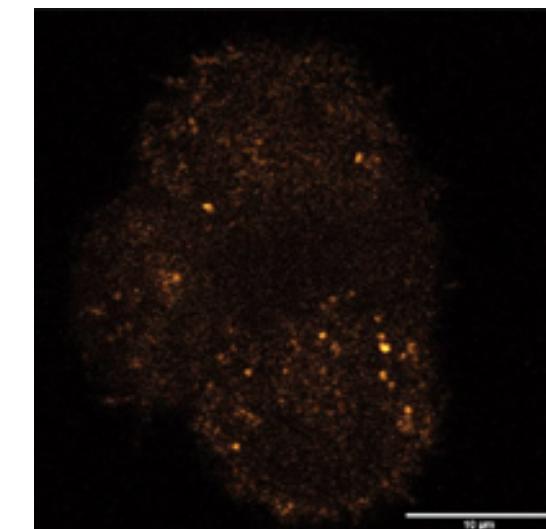
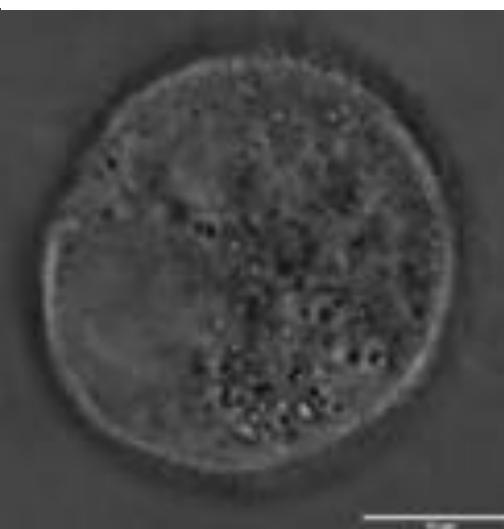
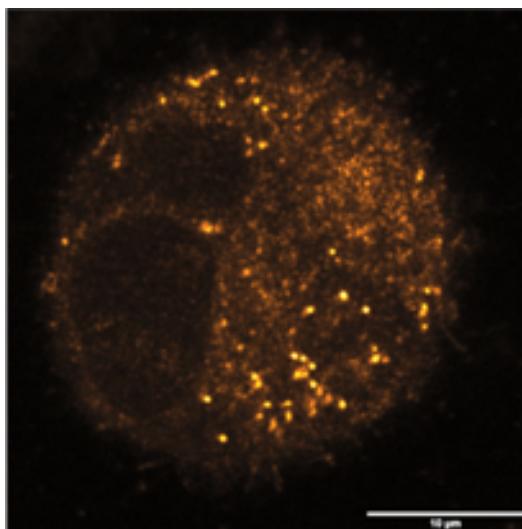
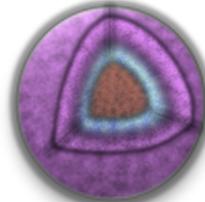
MSNPs



PIG-based hydrogel



HAPEI-MSNPs



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Drs. Monica Ricci

Prof. Dr. Johan Hofkens

Prof. Dr. Hiroshi Uji-i

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Prof. Dr. Paul Kouwer

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Dr. Rodrigo Barderas

Drs. Guillermo Solis

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