



Materials in medicine

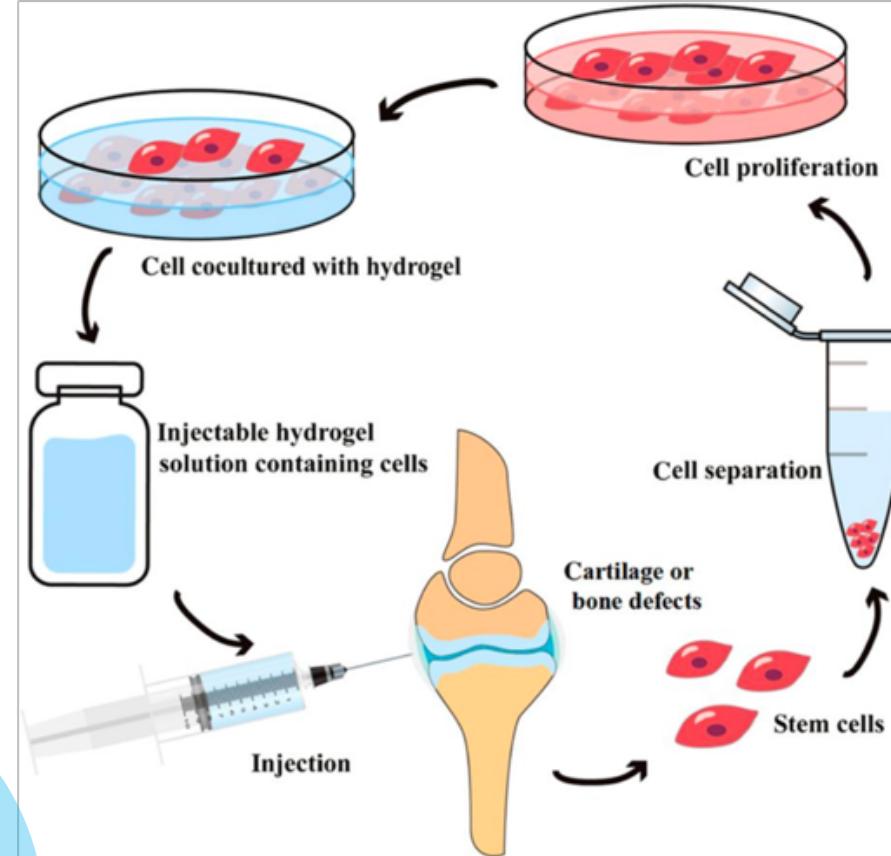
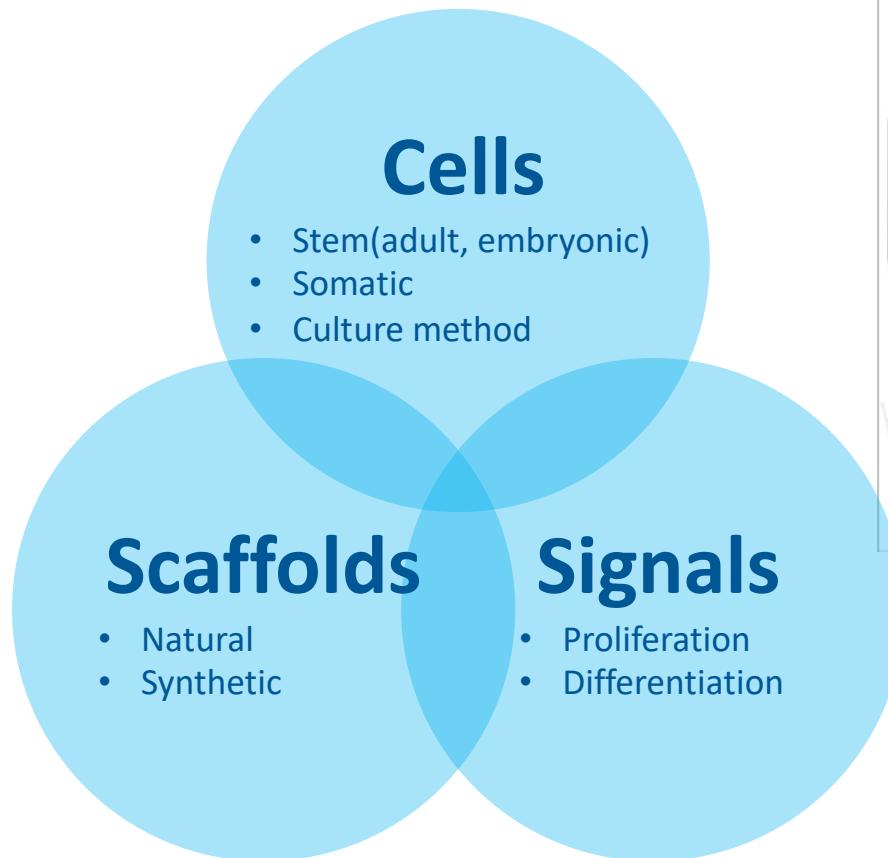
- 1. Biomimetic materials (microscopic characterization)*
- 2. Nanocarriers and Advanced cancer models*

Susana Rocha

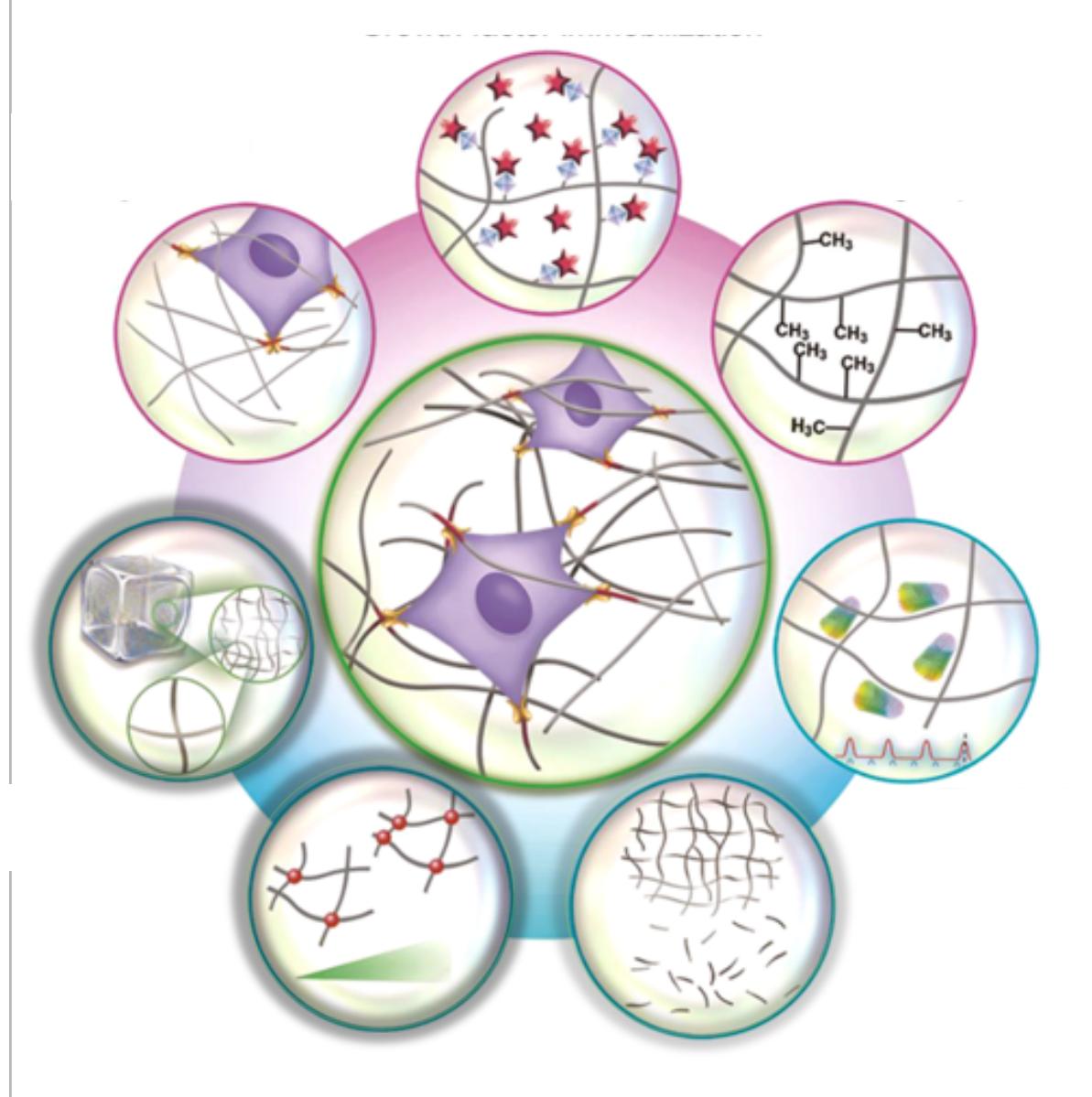
post-doctoral researcher
@ KU Leuven (BE)

Center for Nuclear Sciences and Technologies
18th June 2019

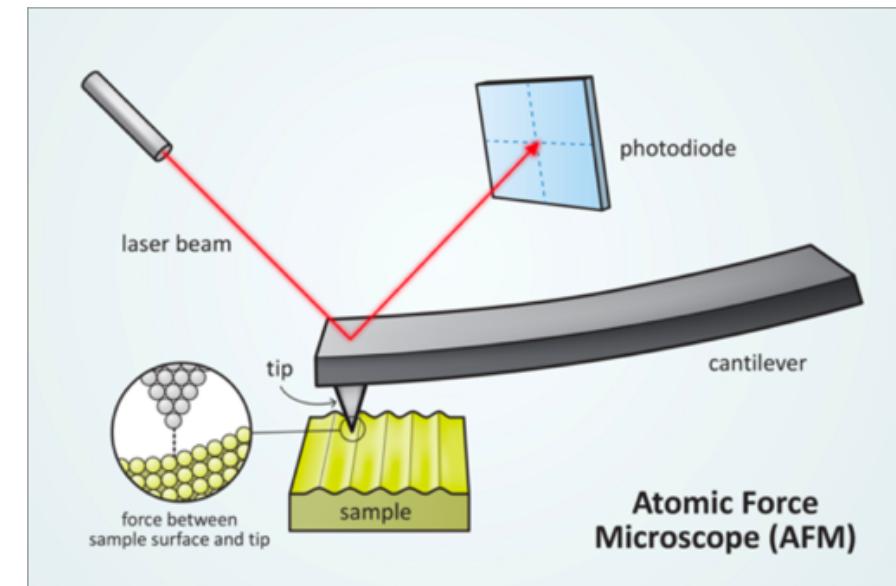
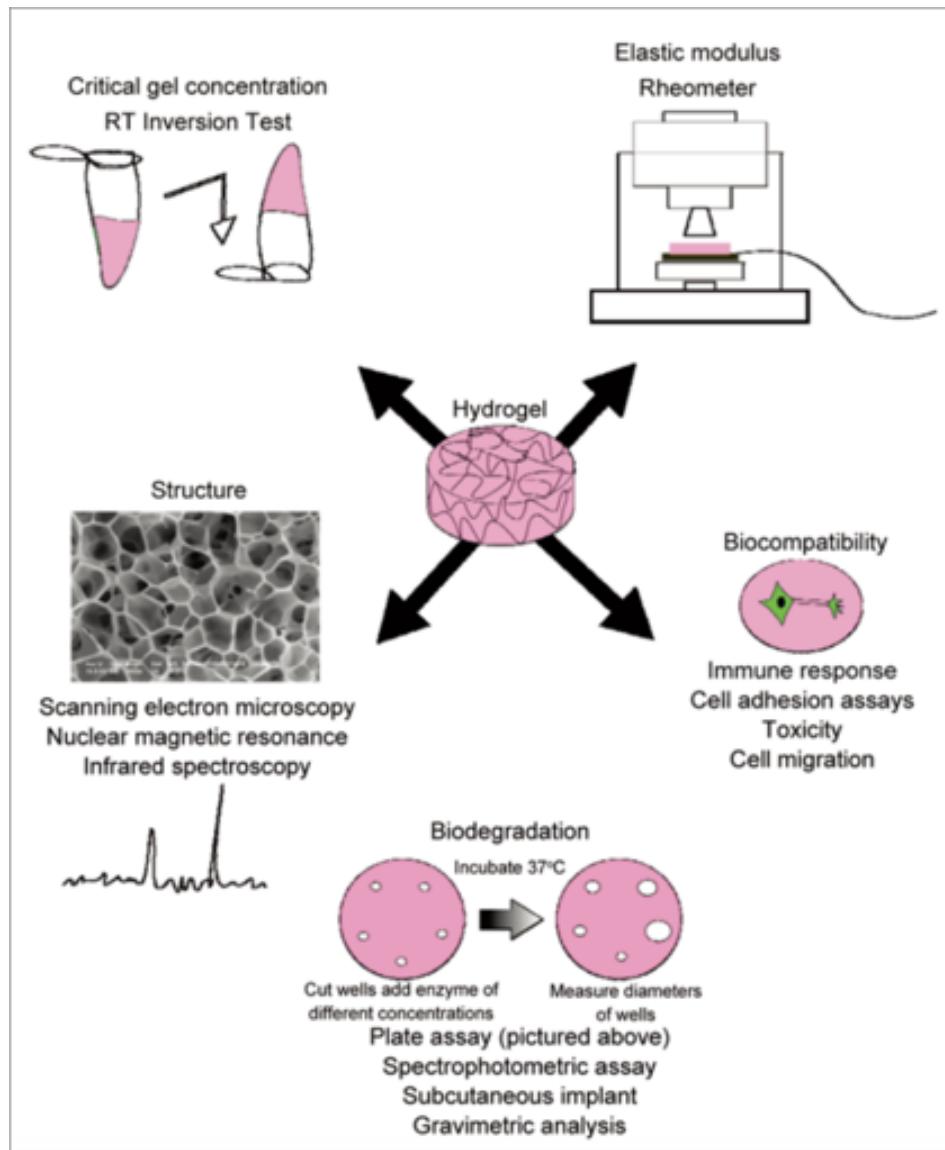
Tissue engineering and 3D cell culture



Design considerations for Biomimetic materials



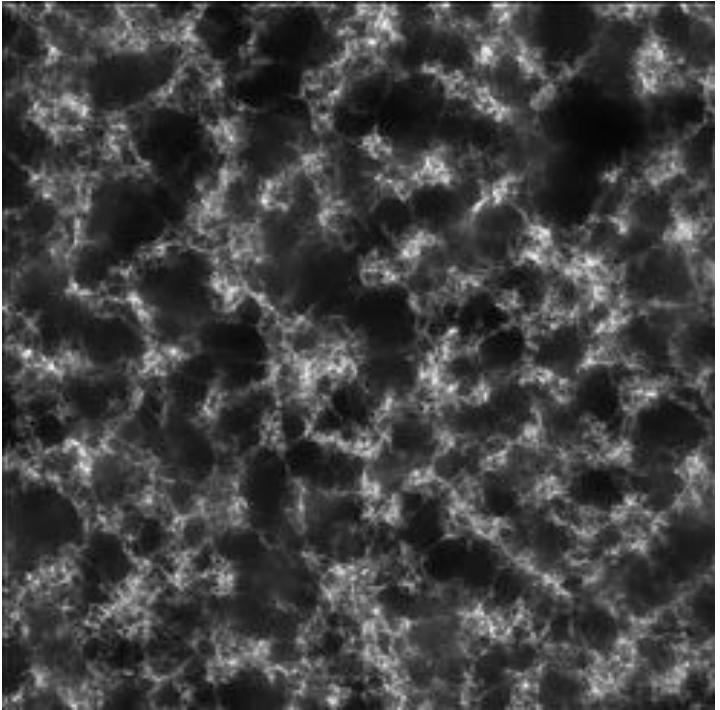
Techniques for characterization of hydrogels



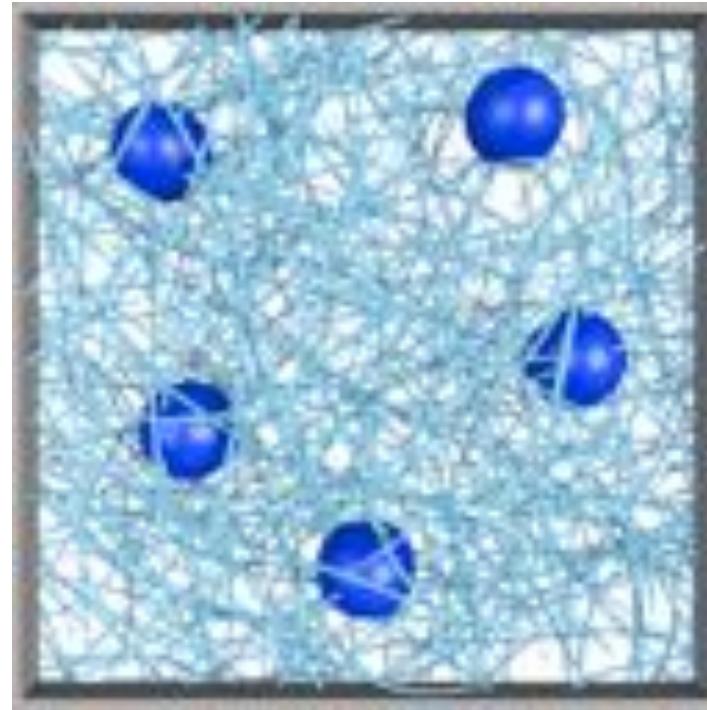
Lack of technologies that can investigate the dynamics of the mechanical and structural properties of biomaterials *in situ*, at the subcellular and molecular level

Material Characterization

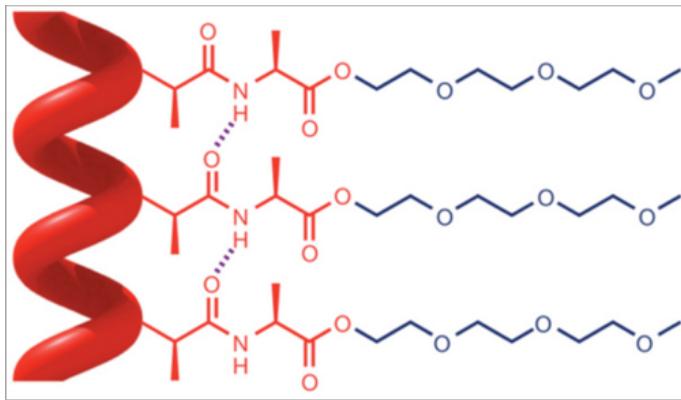
Structure using advanced fluorescence microscopy



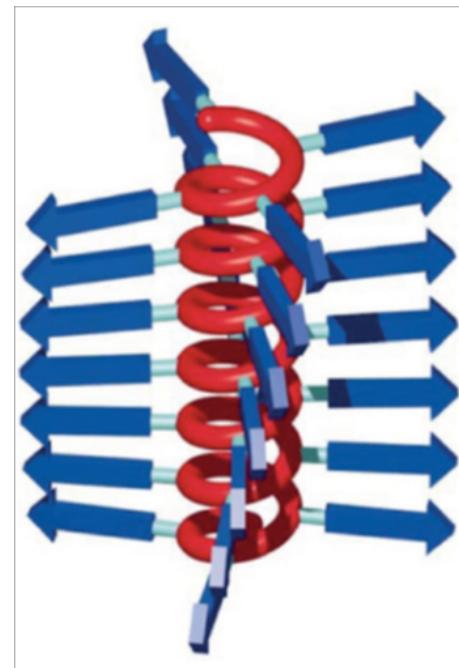
Local mechanical properties using 3D micro-rheology



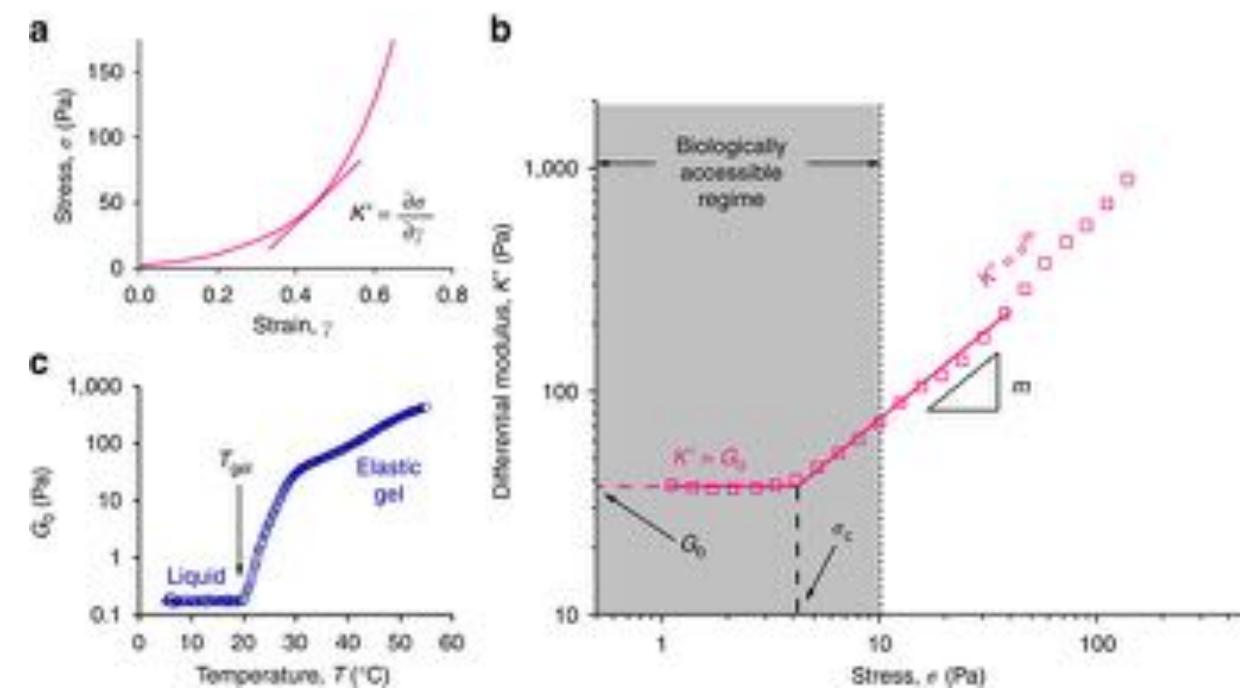
New polymer class for biomimetic 3D matrices



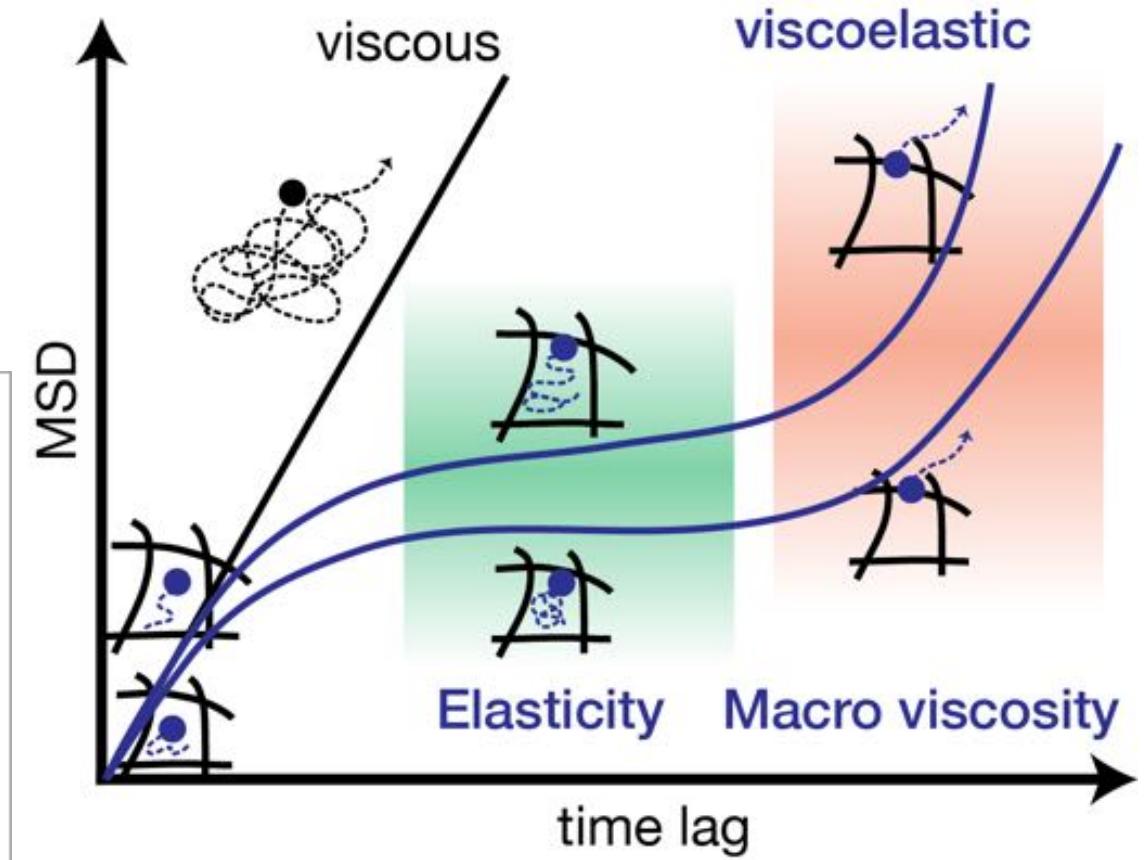
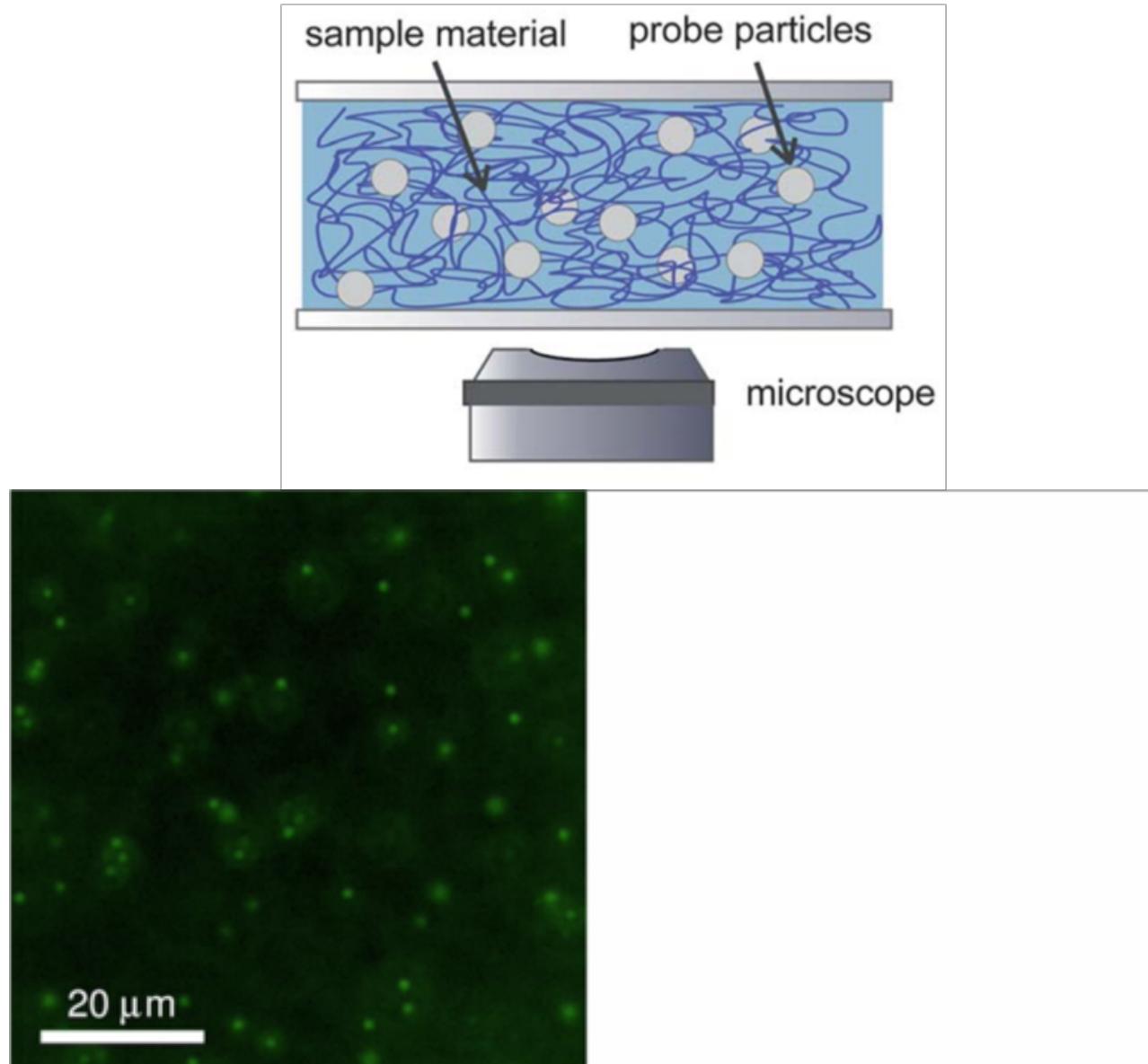
Polyisocyanopeptides (PICs)
grafted with oligo(ethylene glycol)



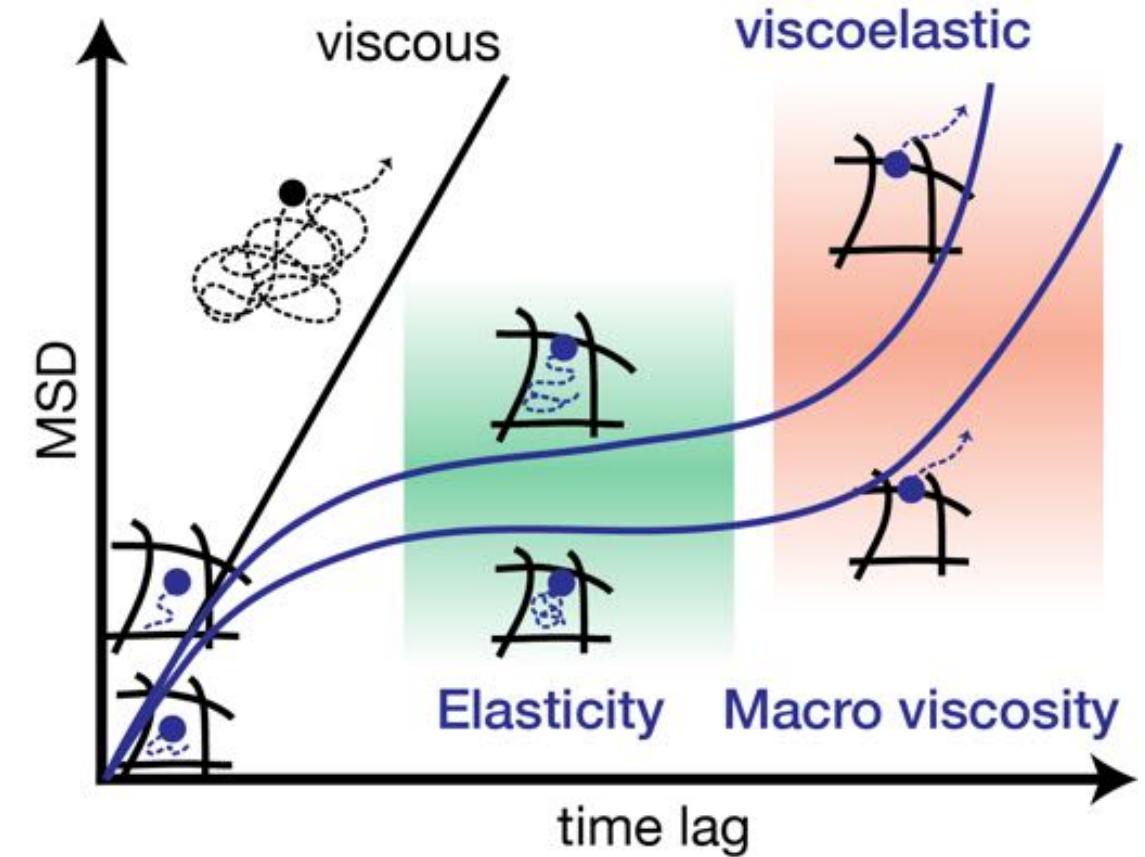
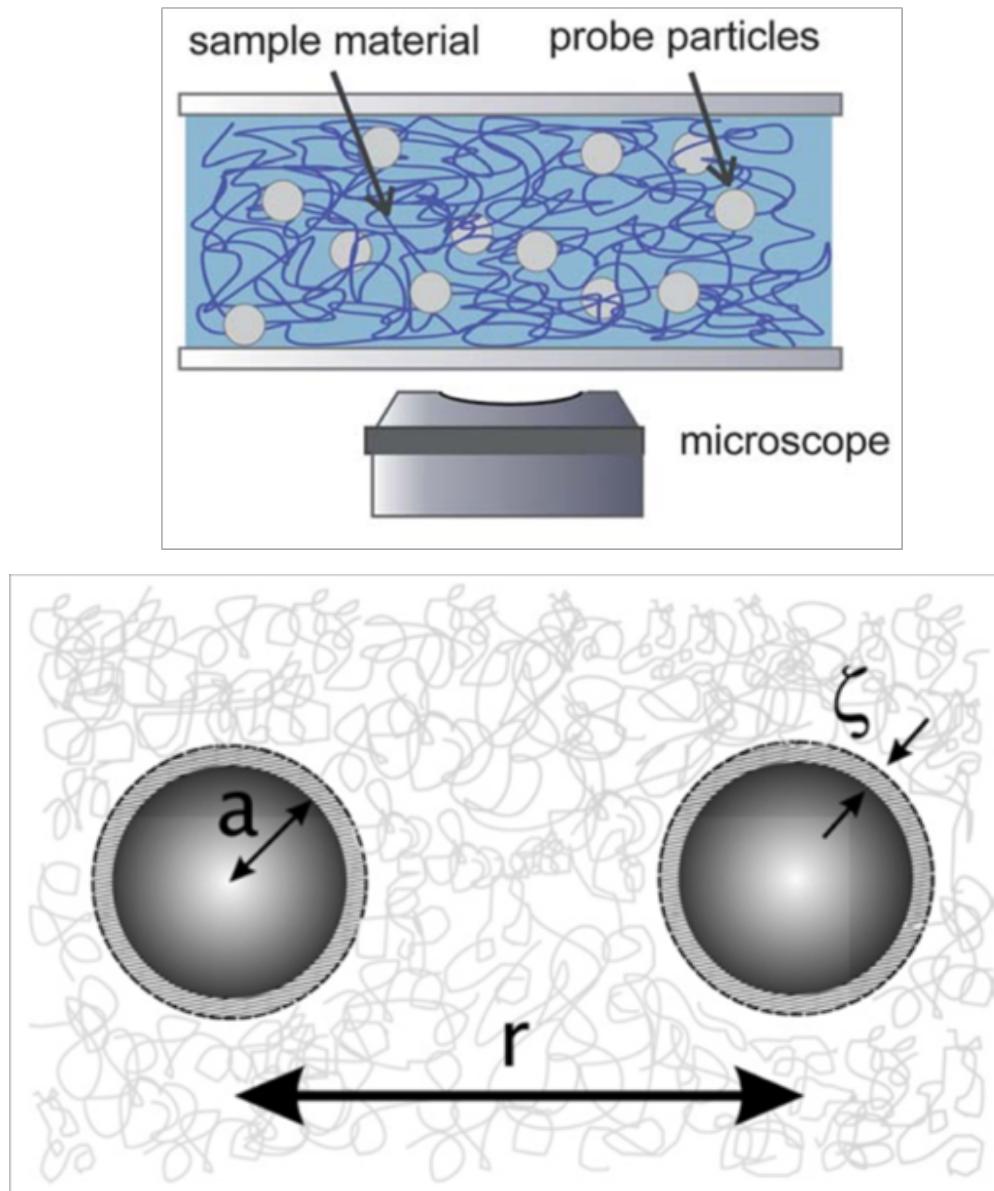
Collaboration with prof. Paul Kouwer,
Radboud University (Nijmegen, The Netherlands)



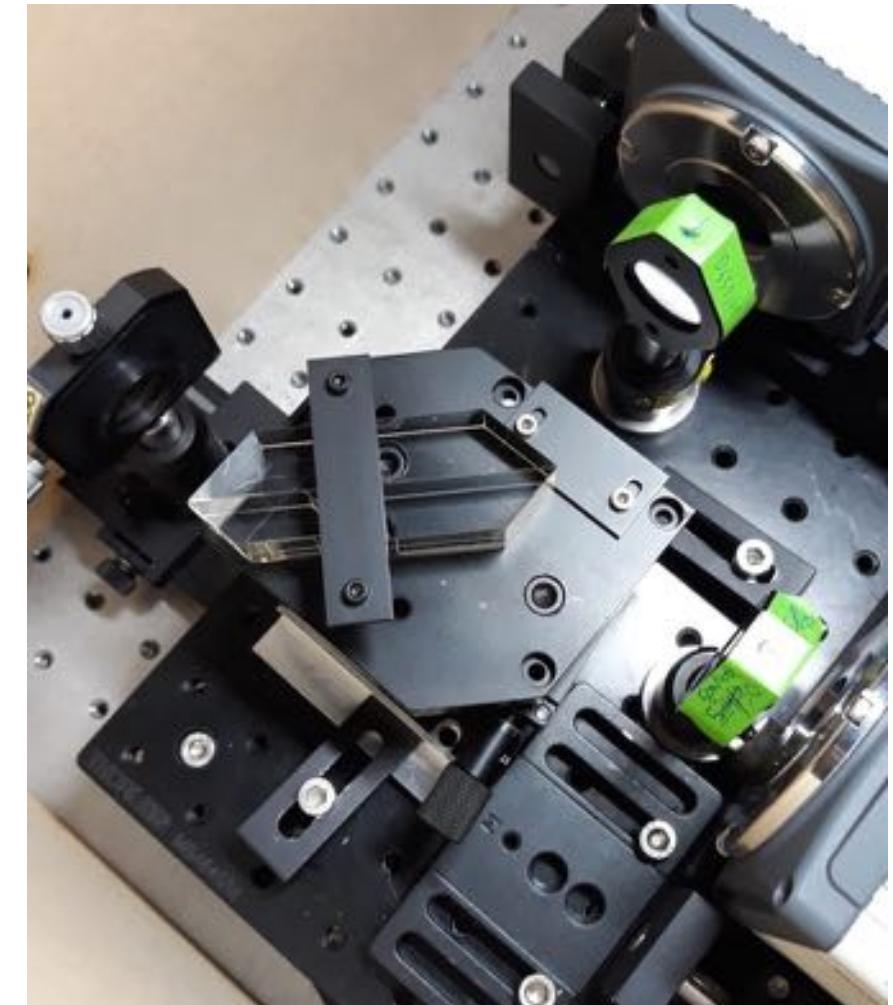
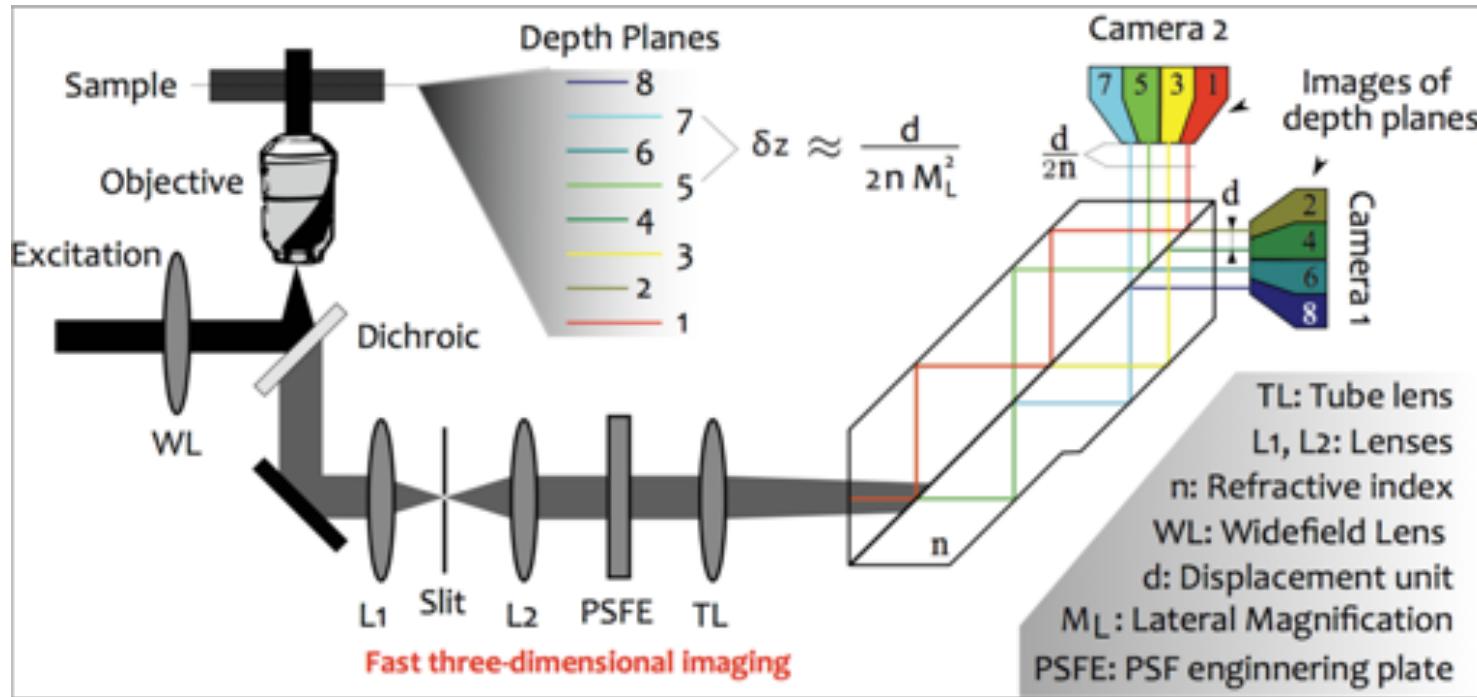
Mechanical Characterization



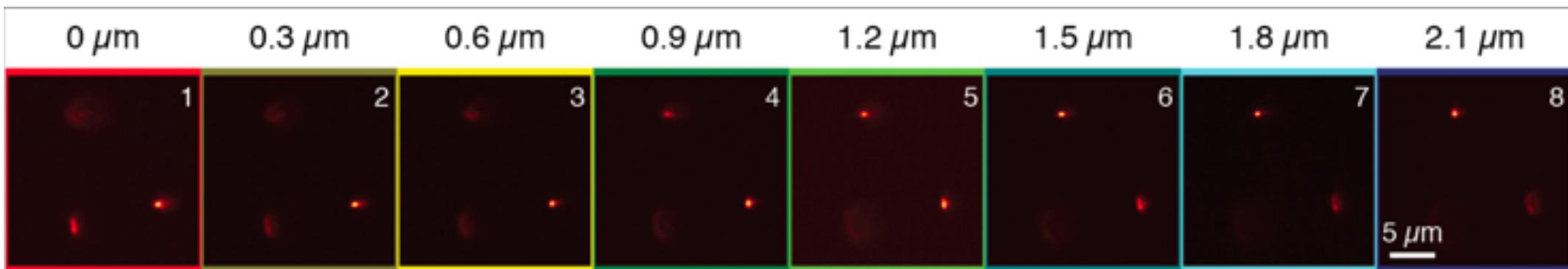
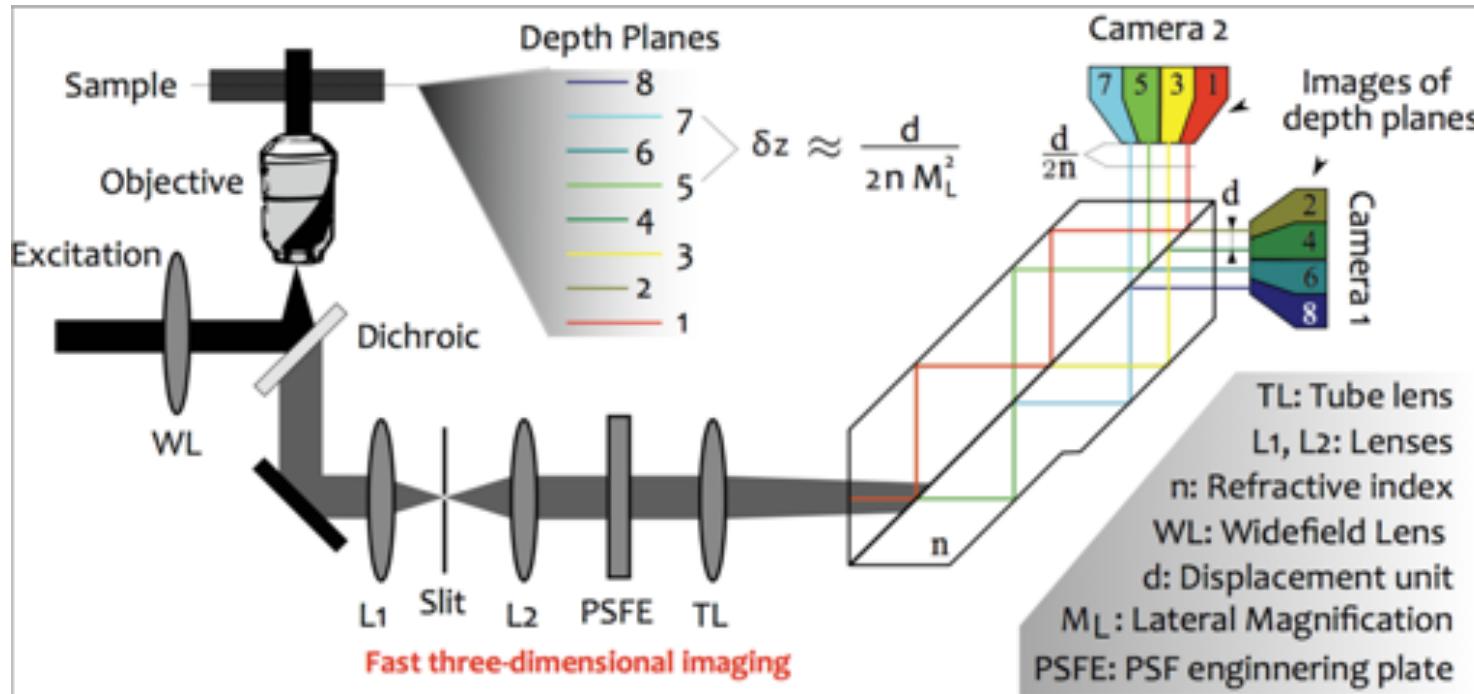
Mechanical Characterization @ the microscale



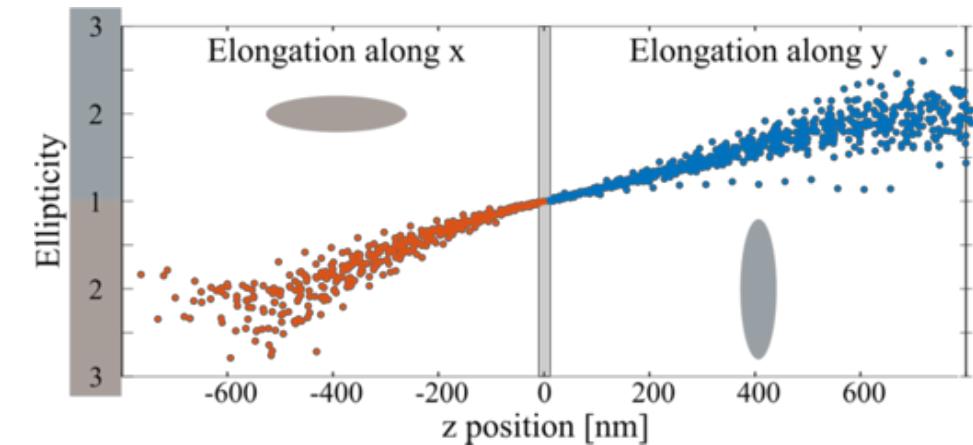
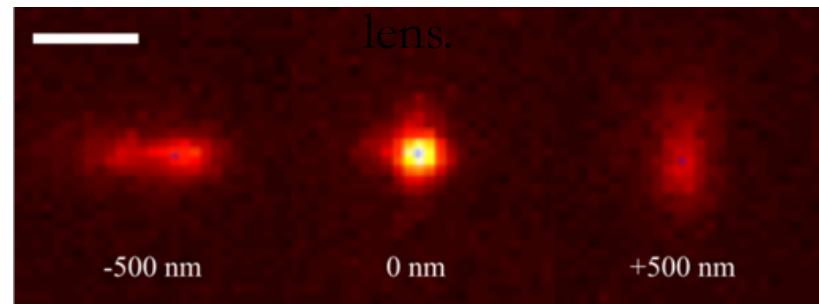
Multi-plane wide field microscope for 2-point micro-rheology



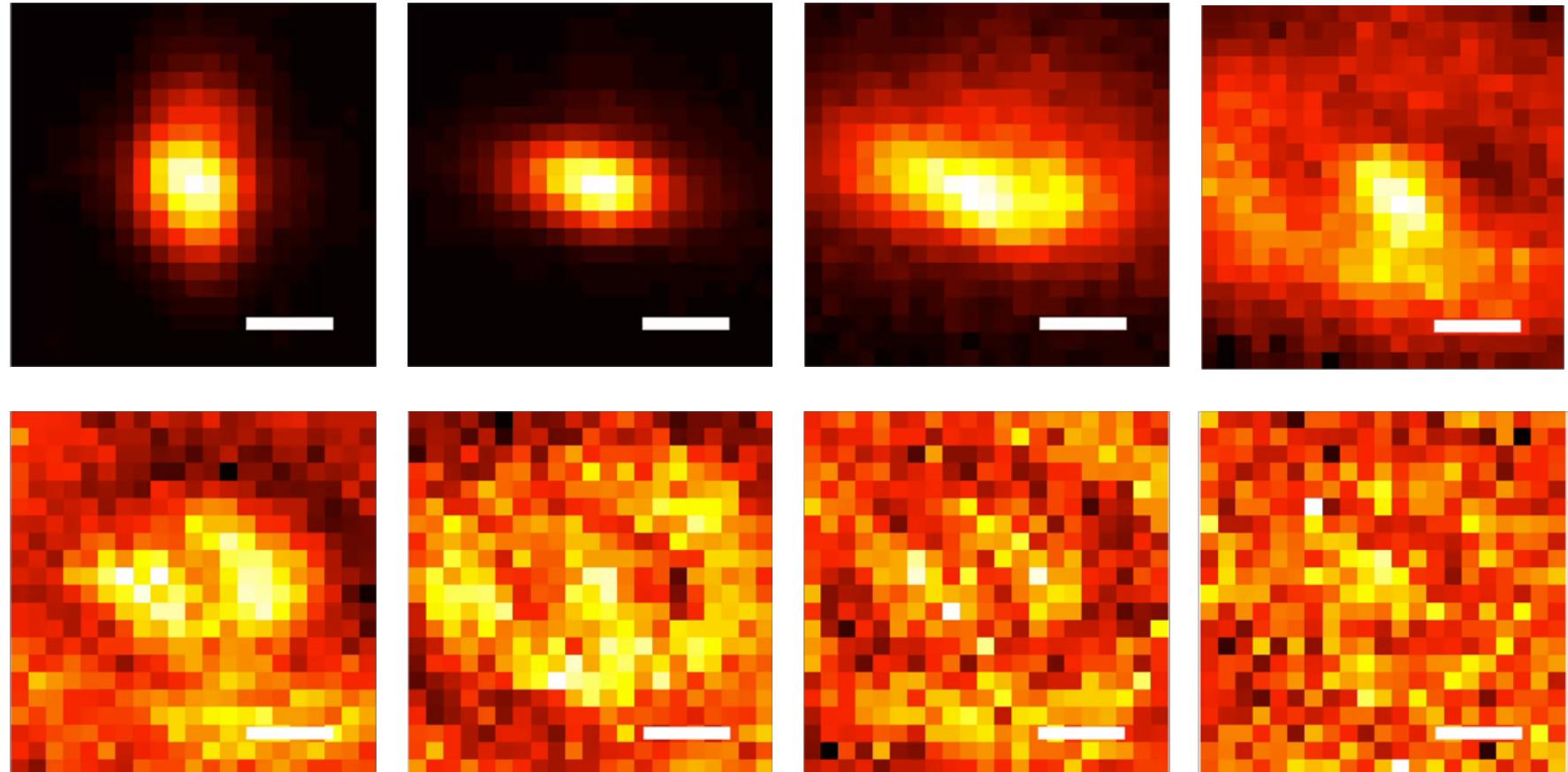
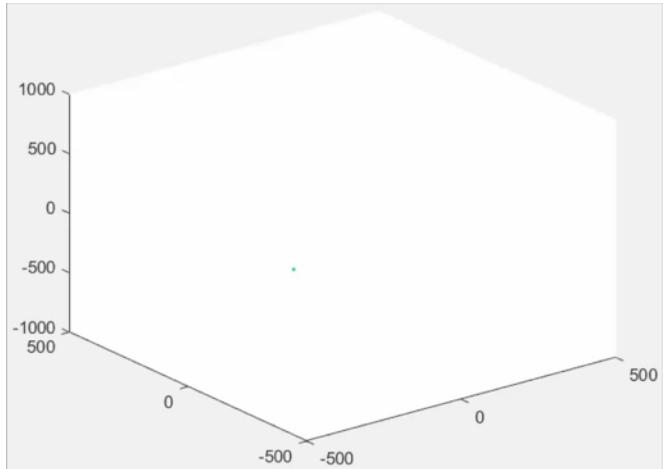
Multi-plane wide field microscope for 2-point micro-rheology



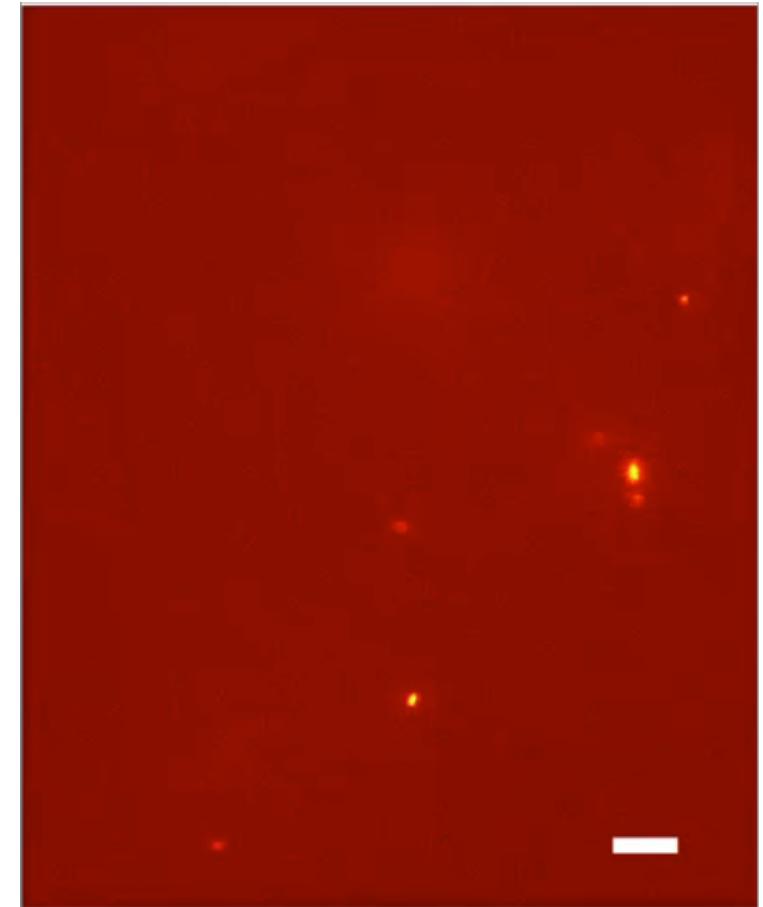
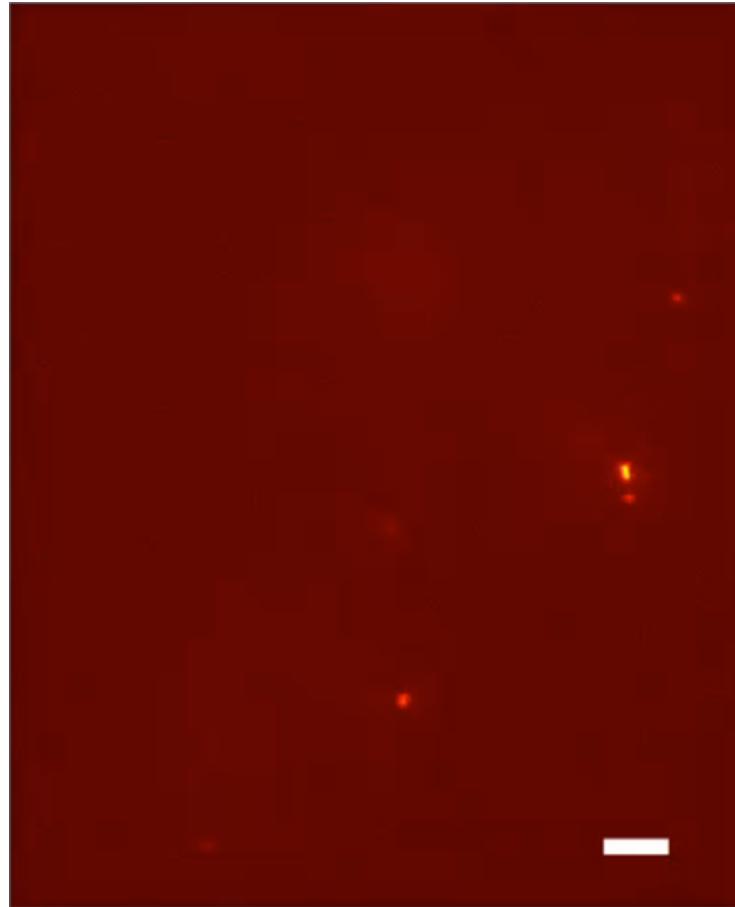
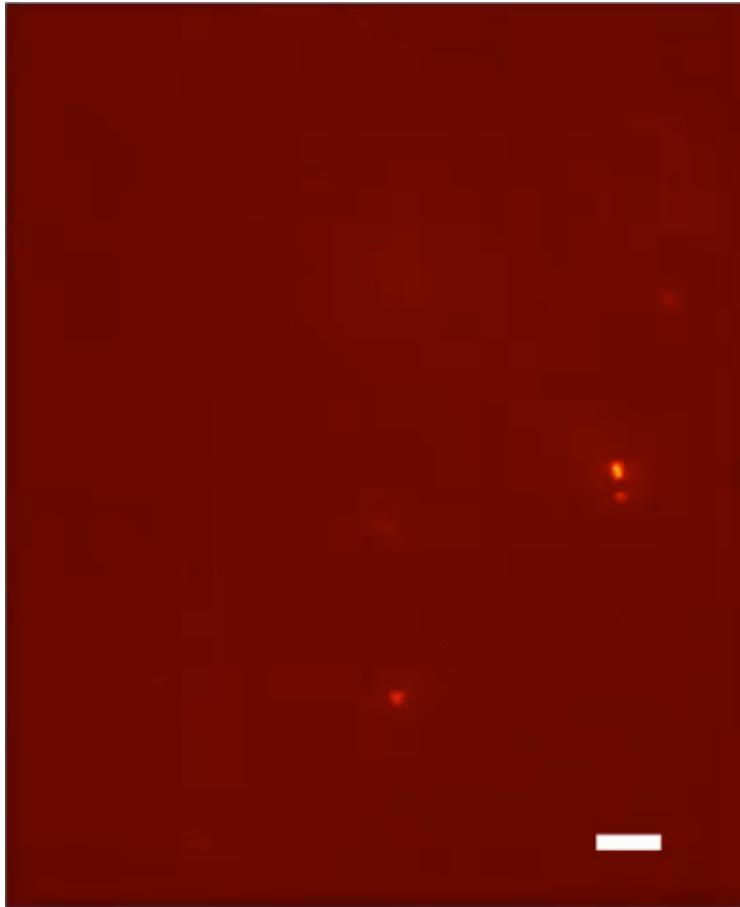
PSF engineering via cylindrical lens.



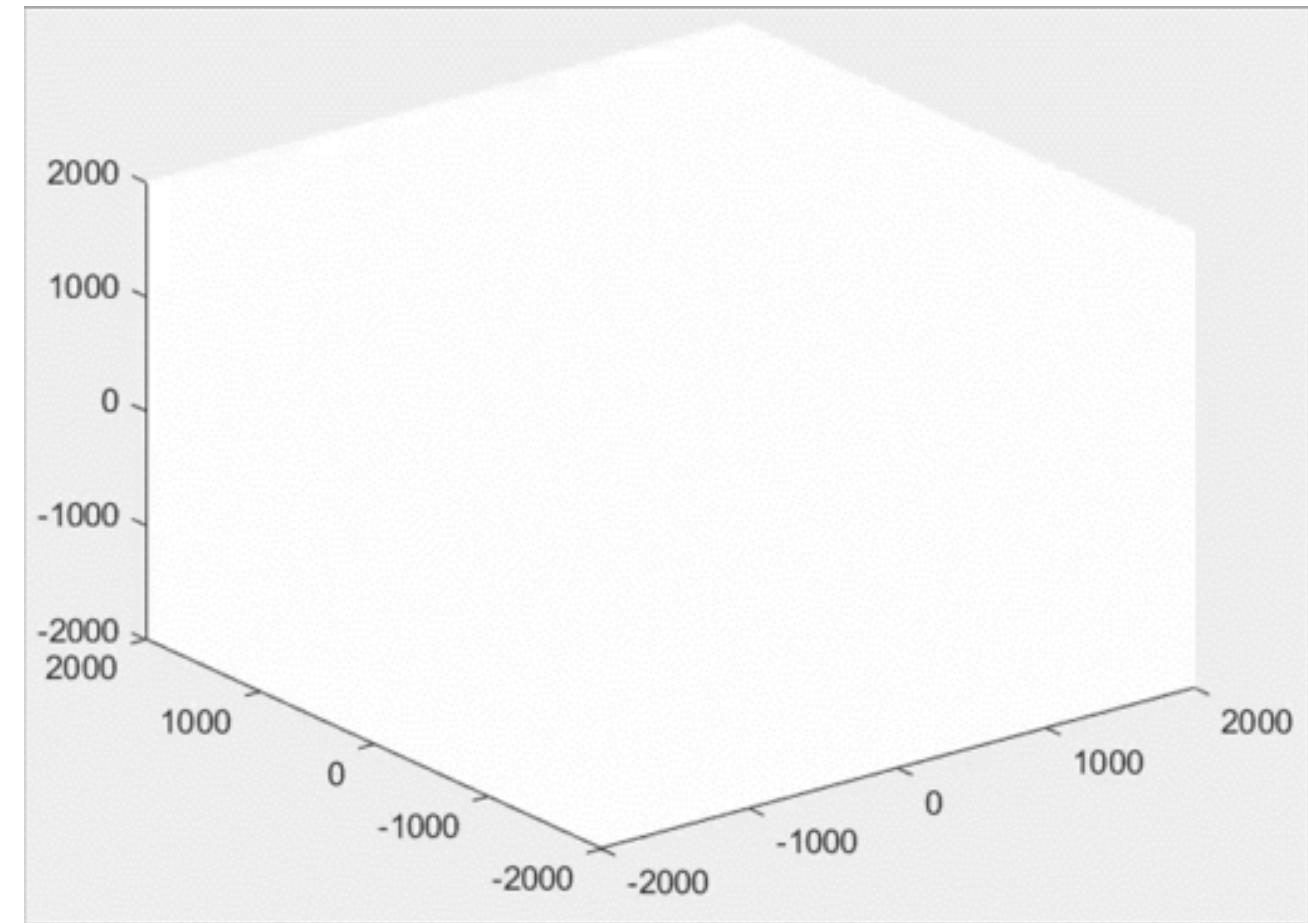
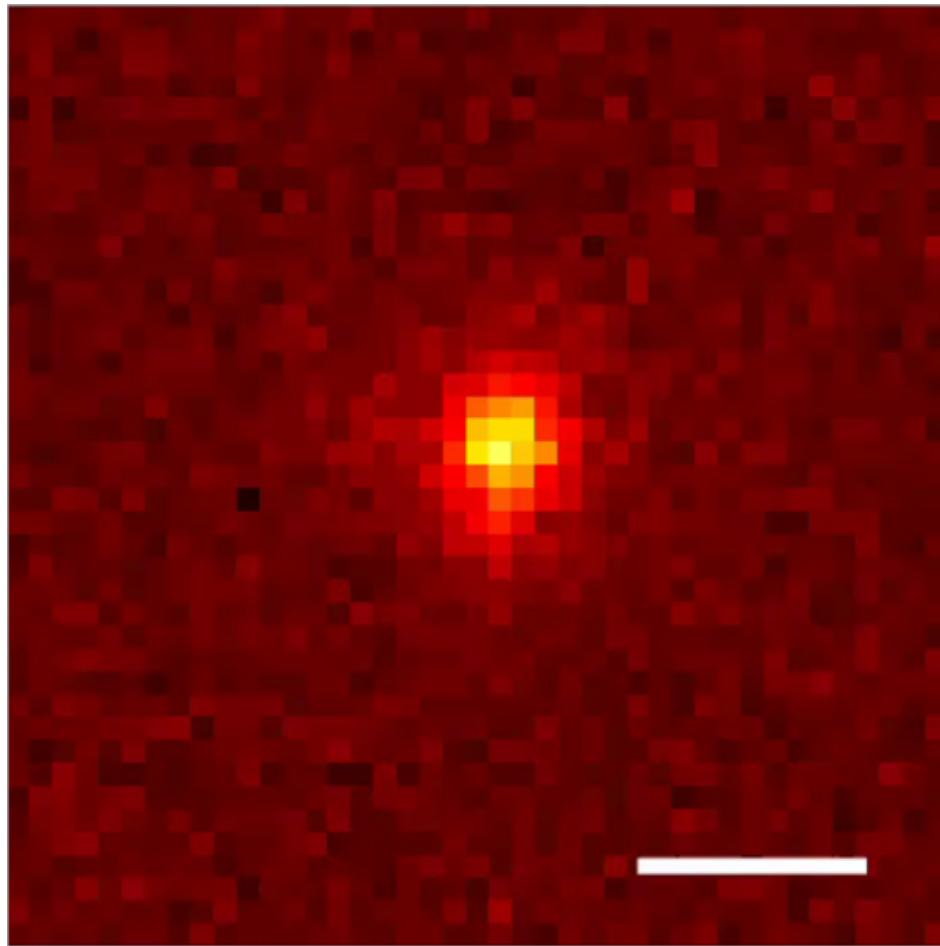
Multi-plane wide field microscope for 2-point micro-rheology



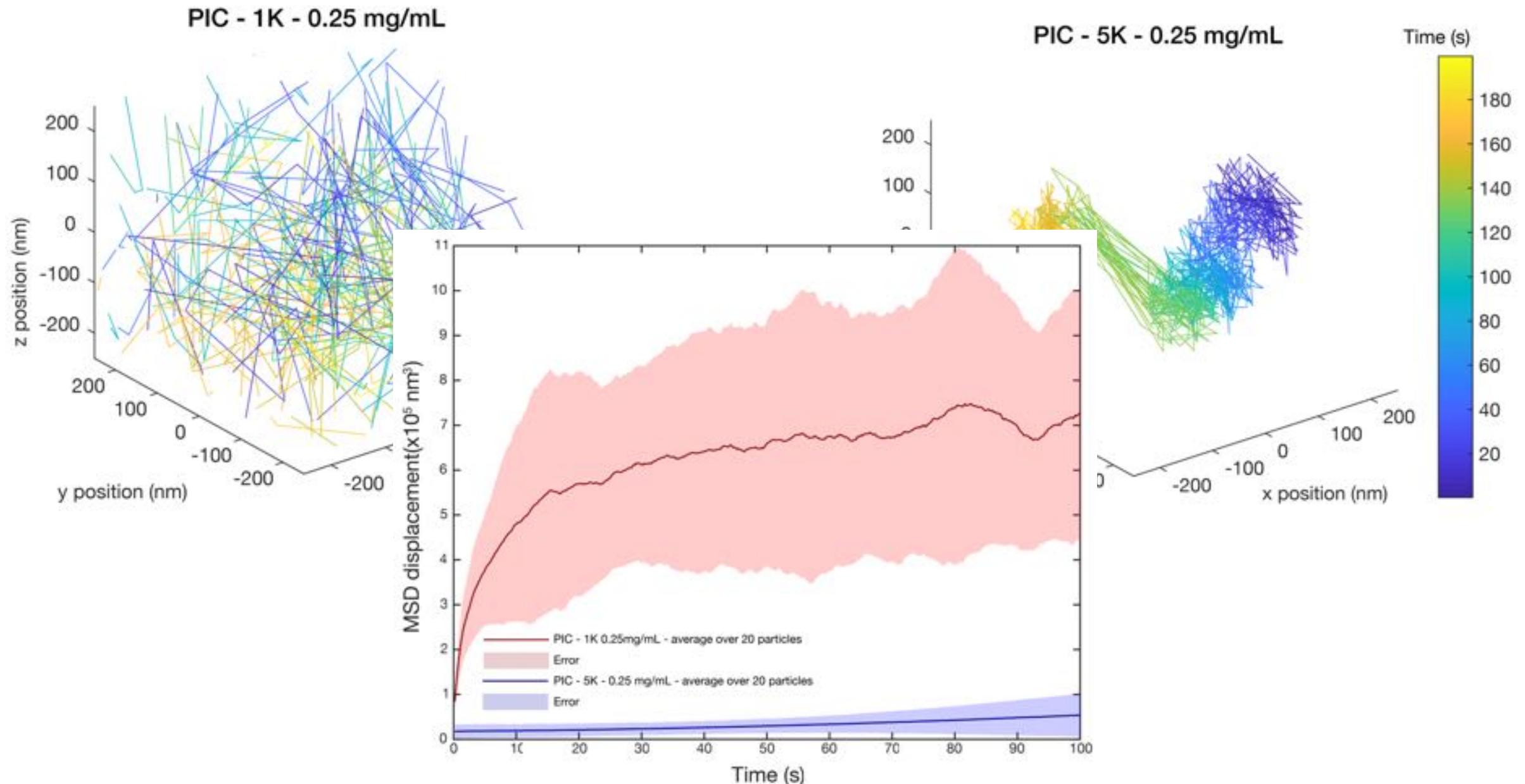
3D SPT in PIC hydrogel



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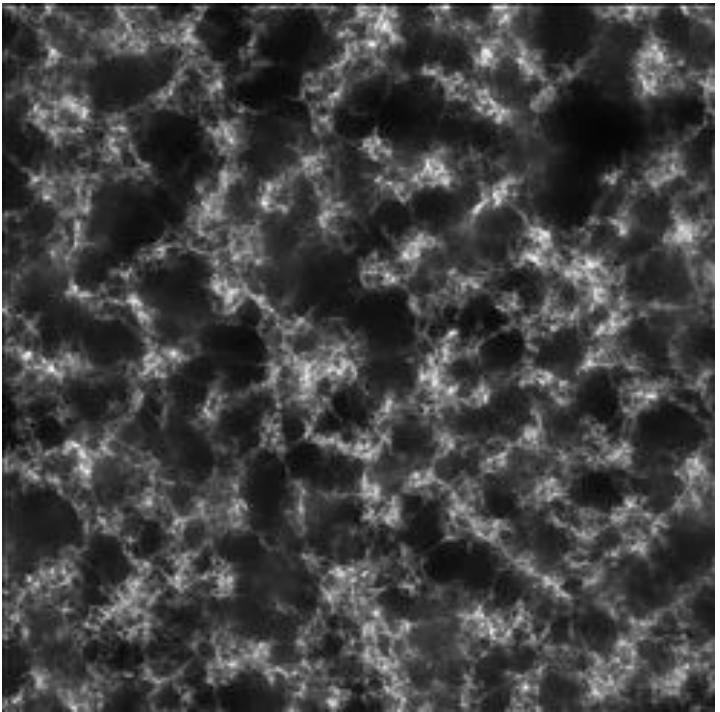


3D SPT in PIC hydrogel

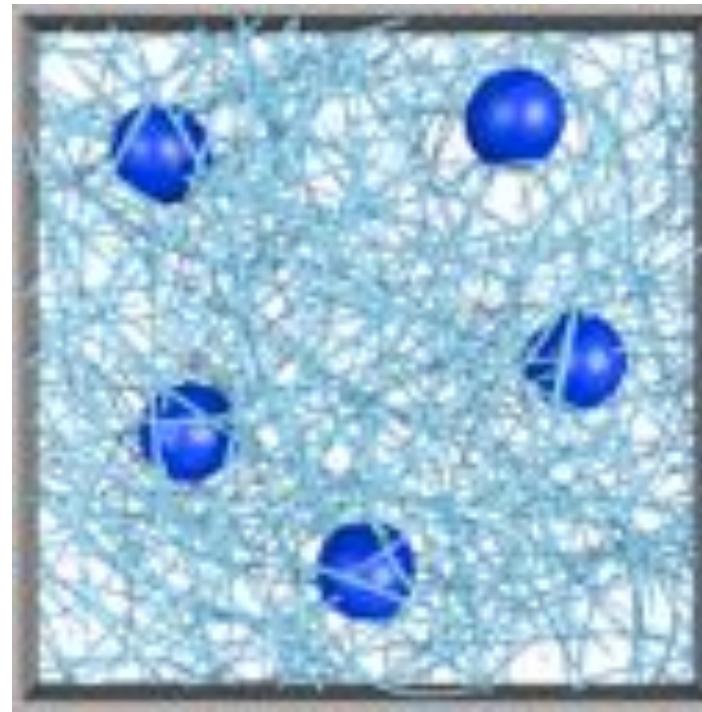


Material Characterization

Structure using advanced fluorescence microscopy

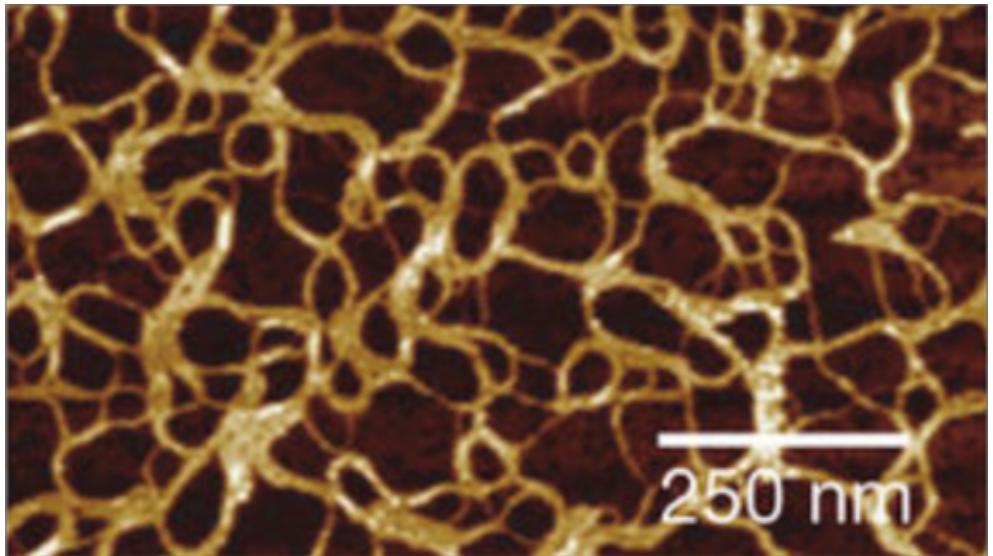


Local mechanical properties using 3D micro-rheology

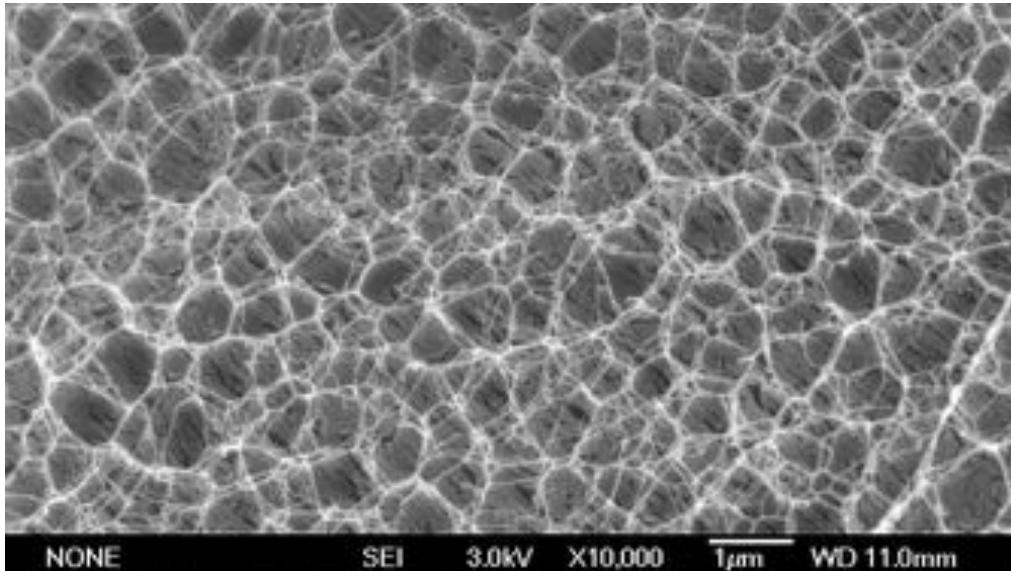


Structural Characterization of PIC-based hydrogels

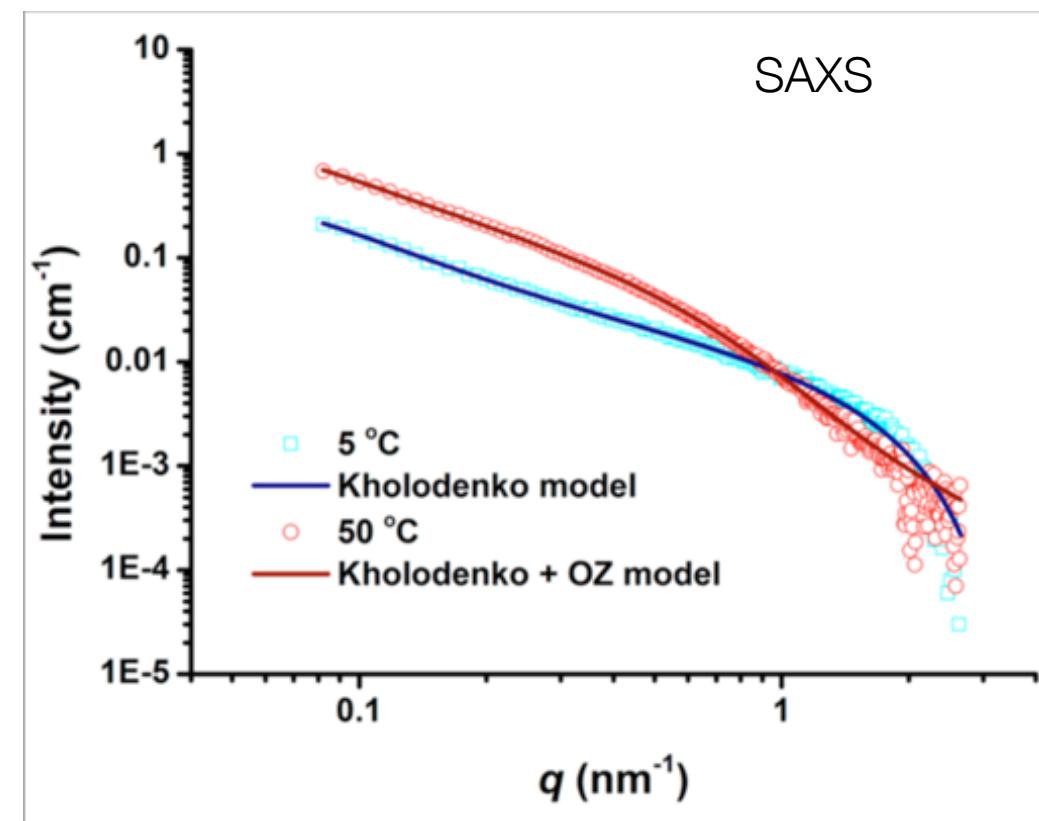
AFM



CryoEM



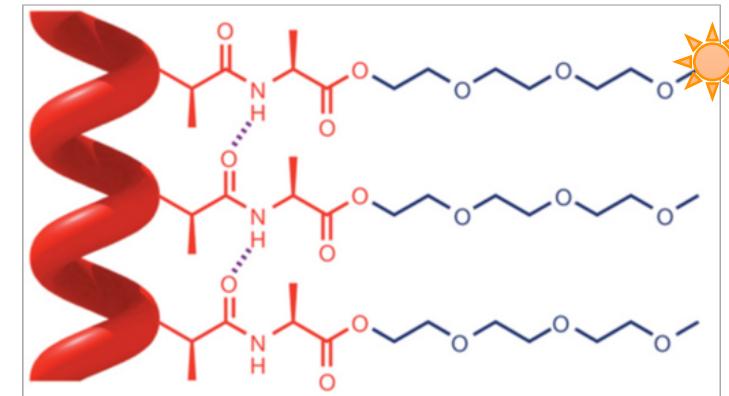
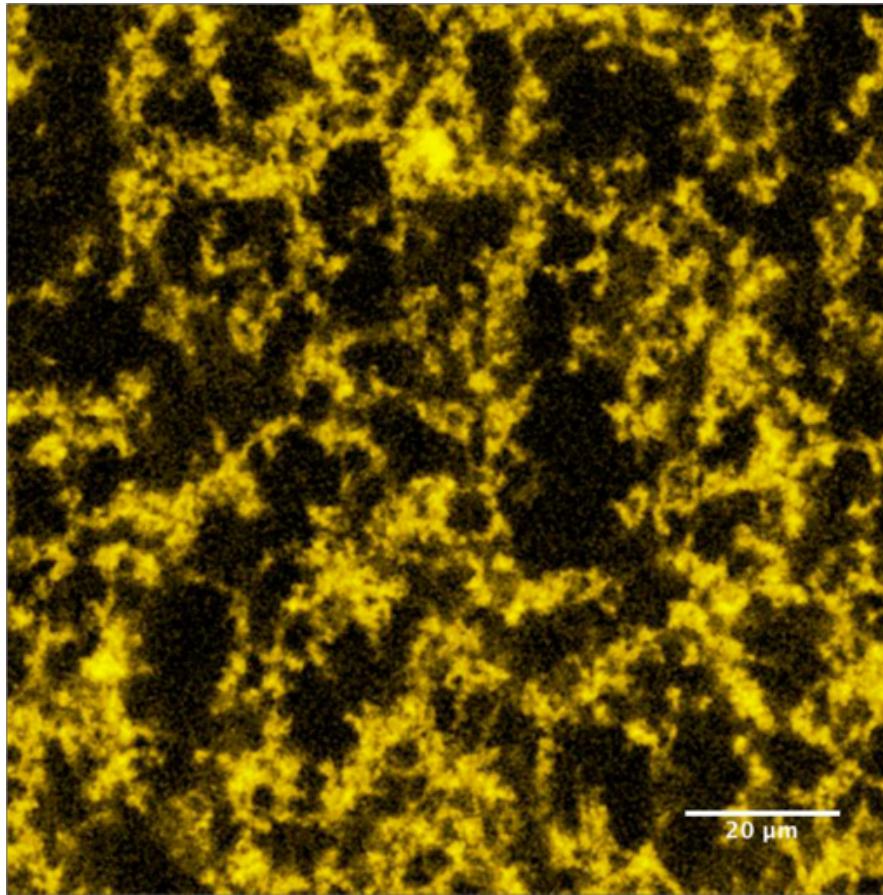
SAXS



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

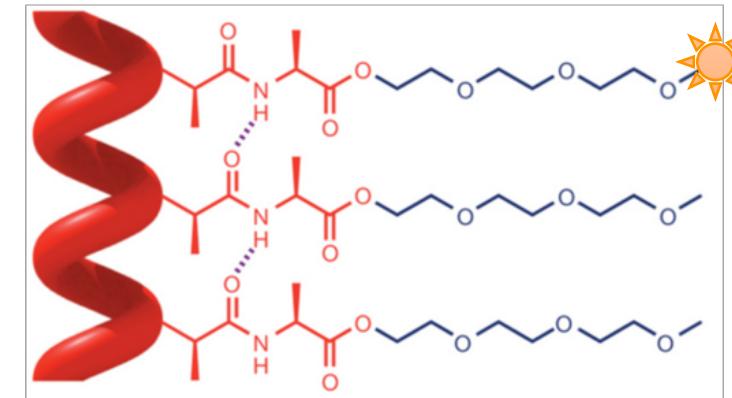
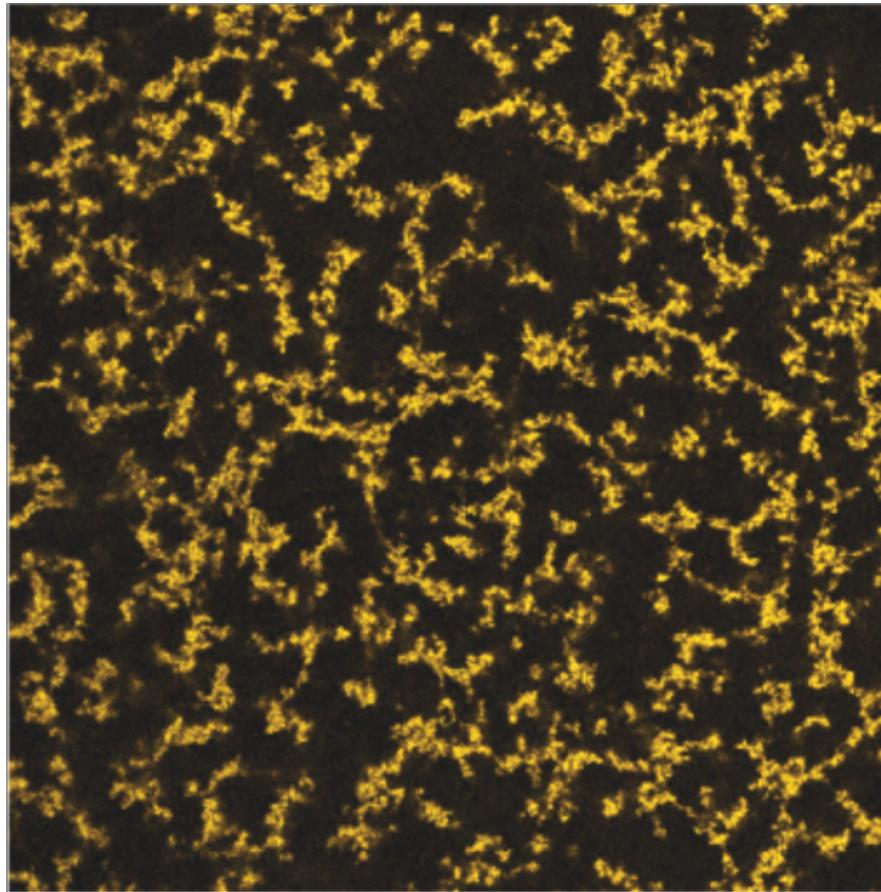
Structural Characterization of PIC-based hydrogels

Fluorescence Microscopy



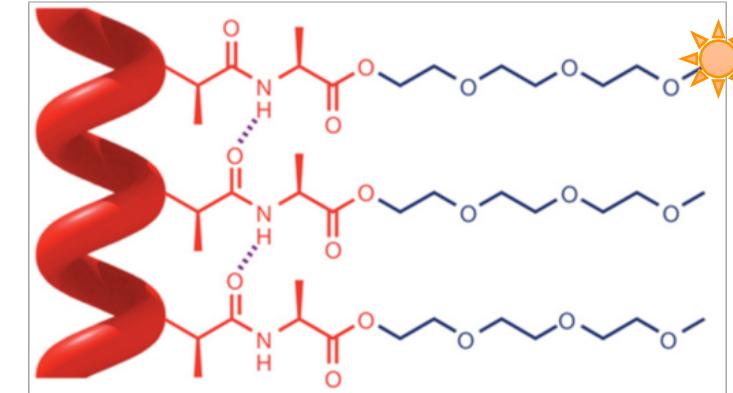
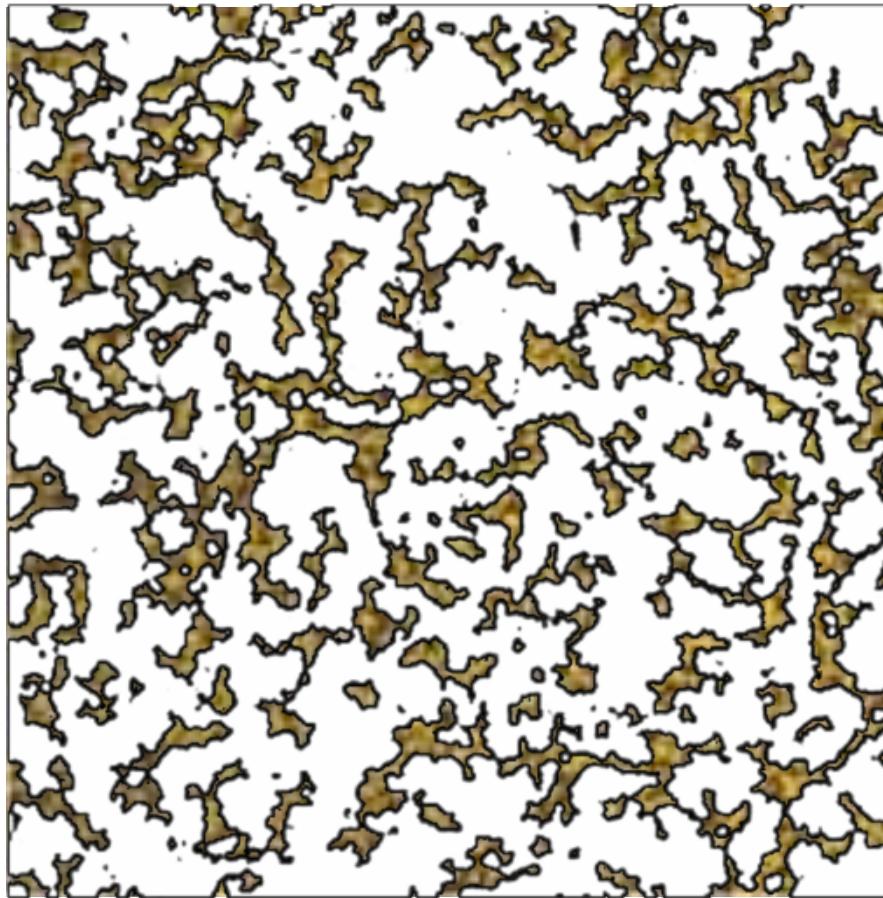
Structural Characterization of PIC-based hydrogels

Fluorescence Microscopy

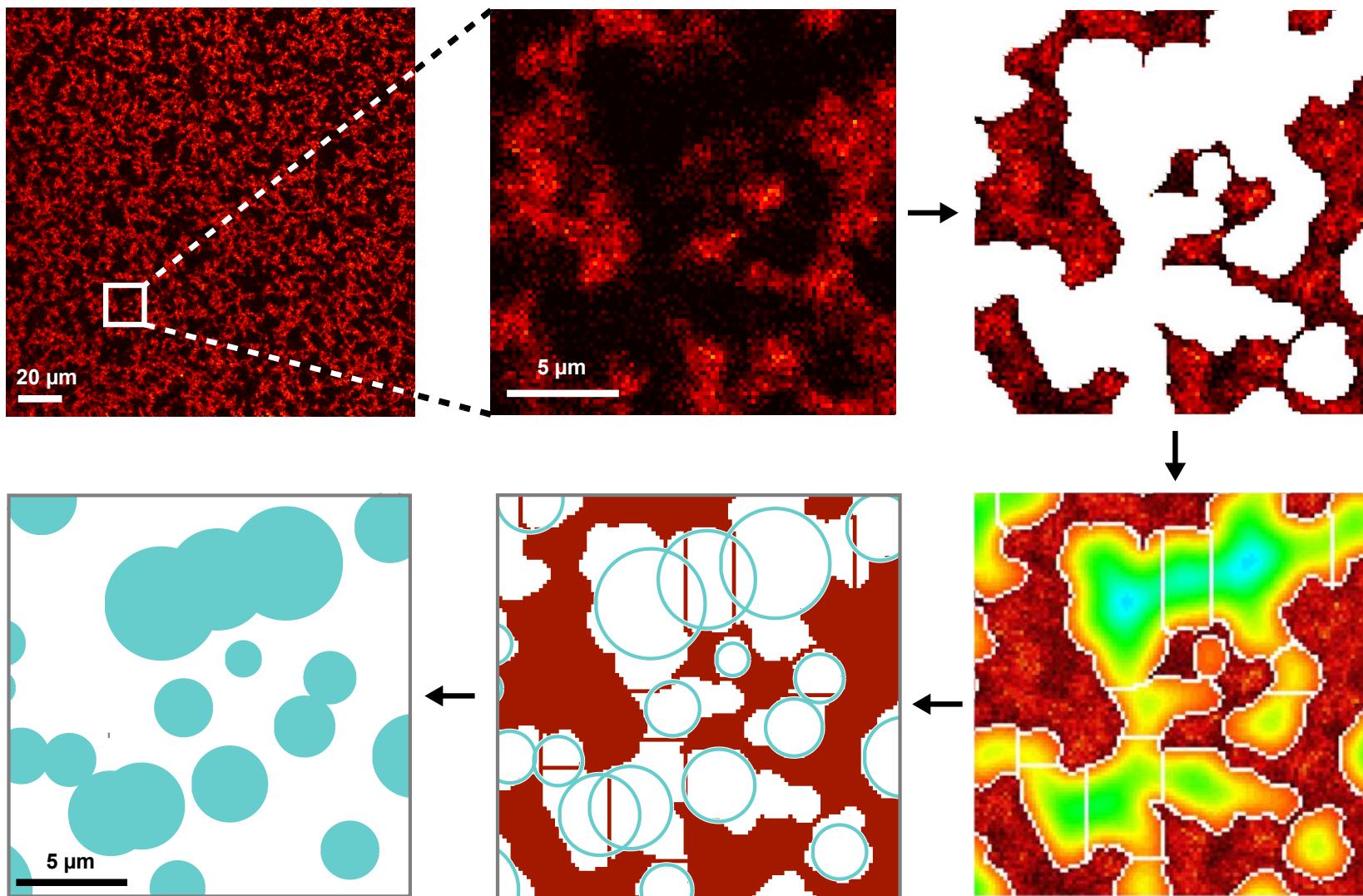


Structural Characterization of PIC-based hydrogels

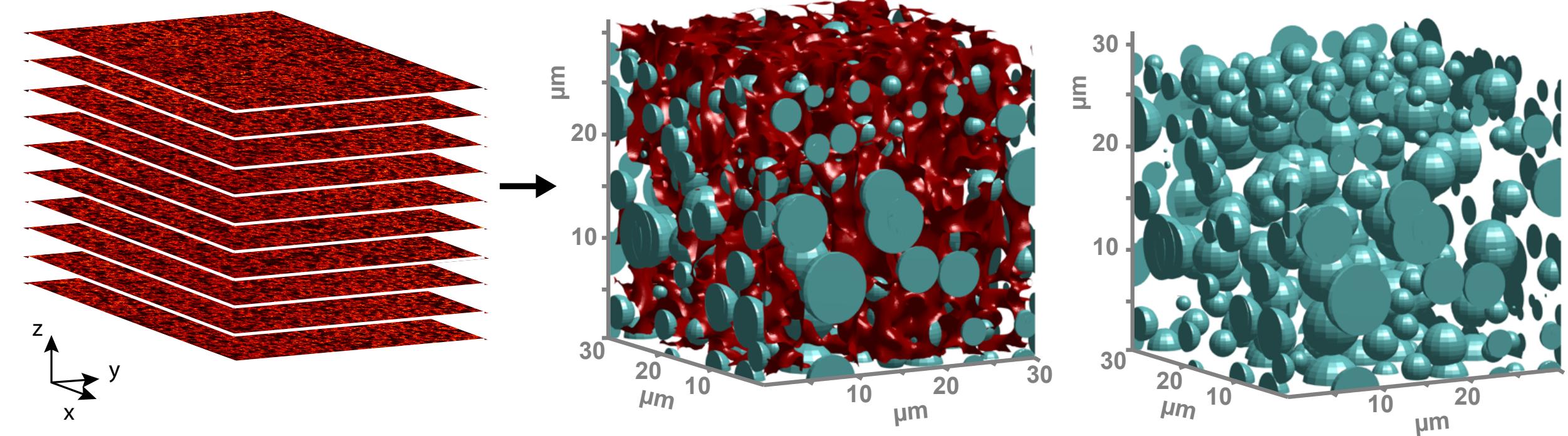
Fluorescence Microscopy



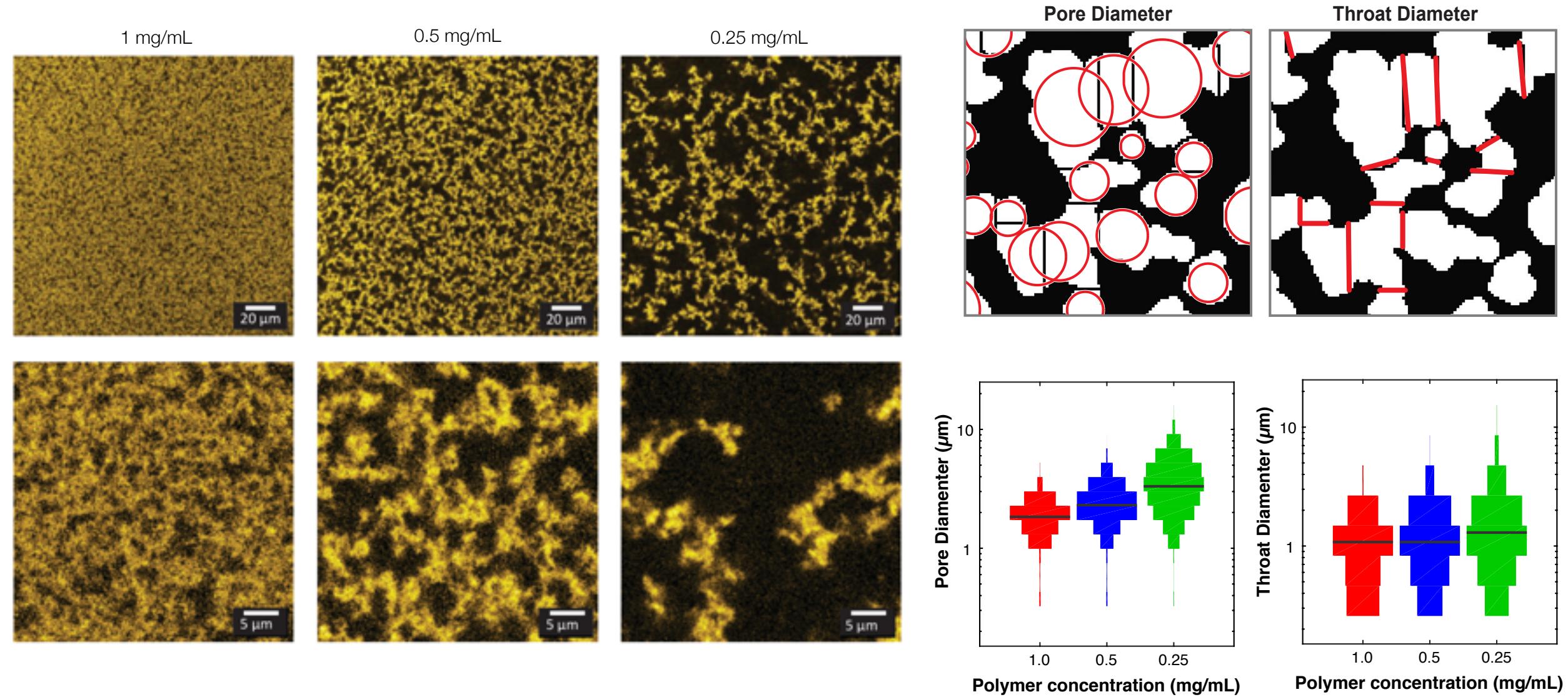
Structural Characterization of PIC-based hydrogels



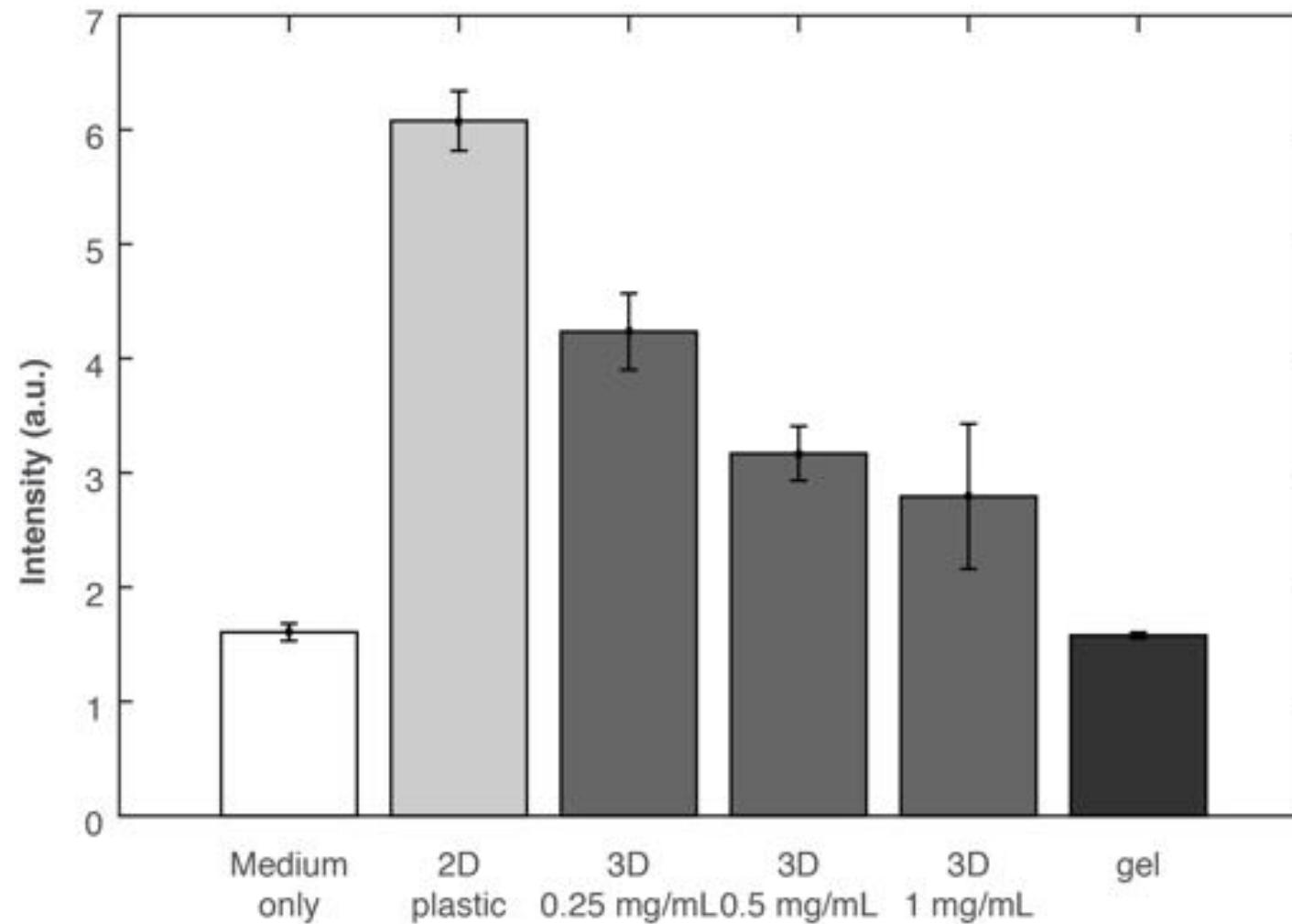
Structural Characterization of PIC-based hydrogels in 3D



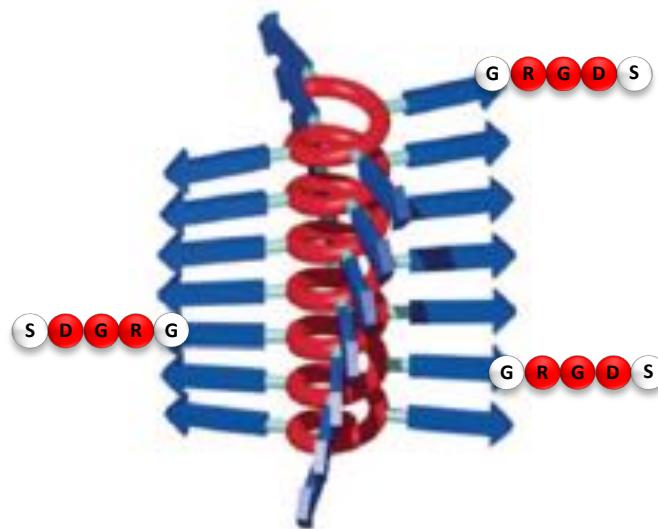
Structural Characterization of PIC-based hydrogels



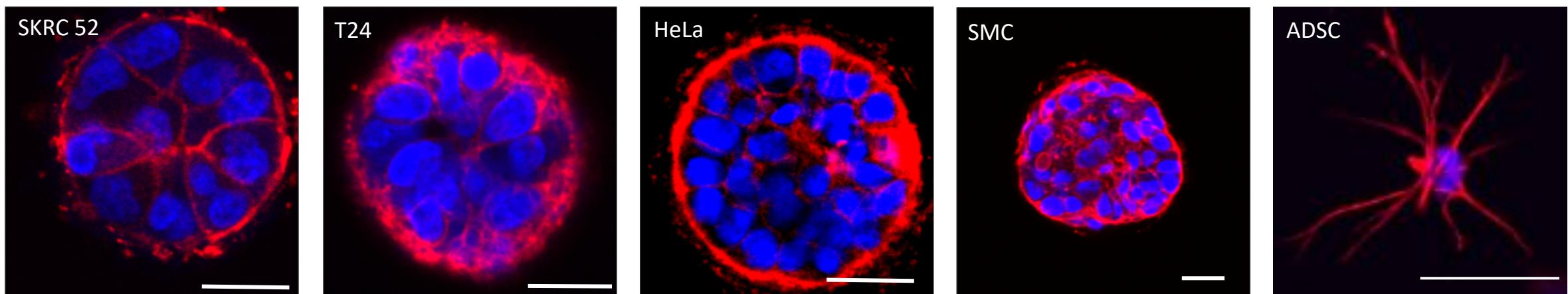
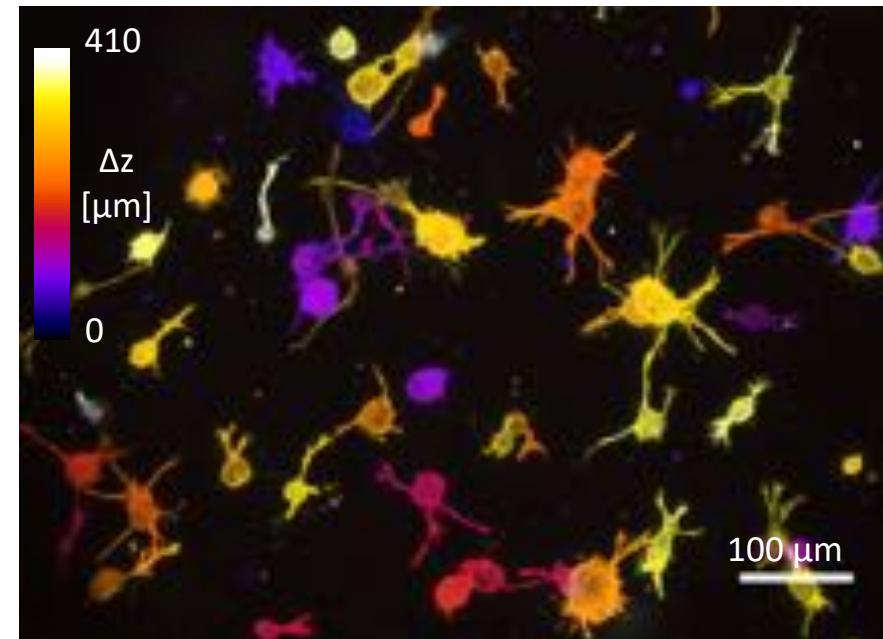
Hydrogel structure and cell proliferation



Minimalist matrix: Polyisocyanopeptides (PIC)

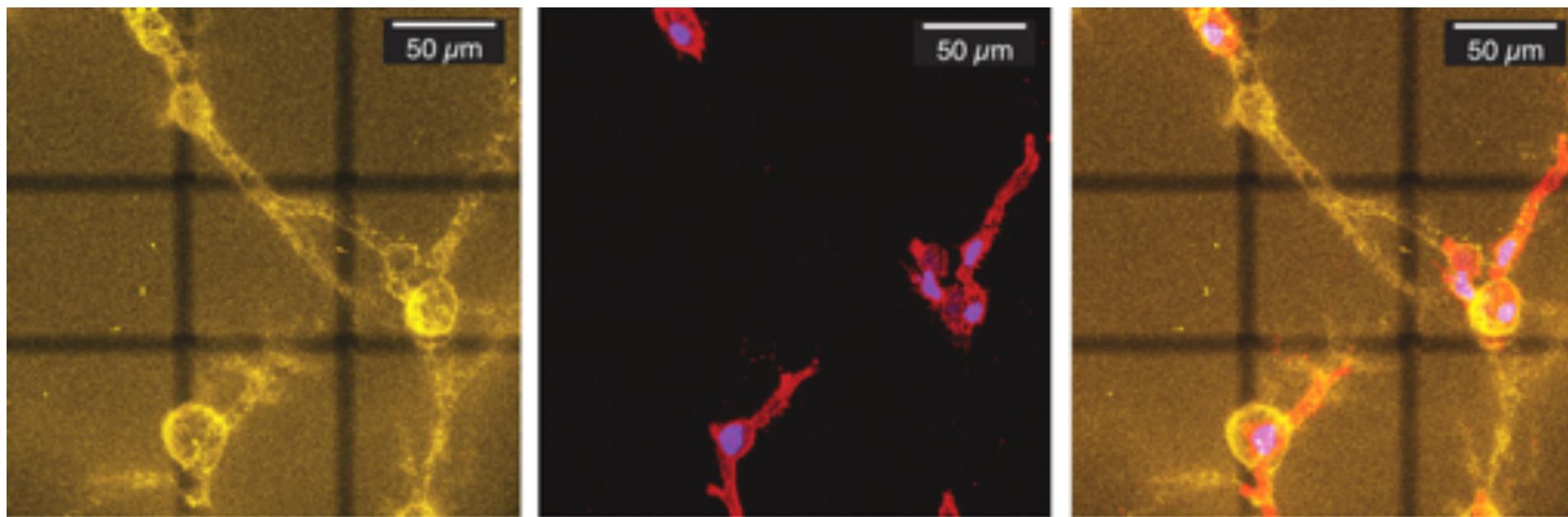
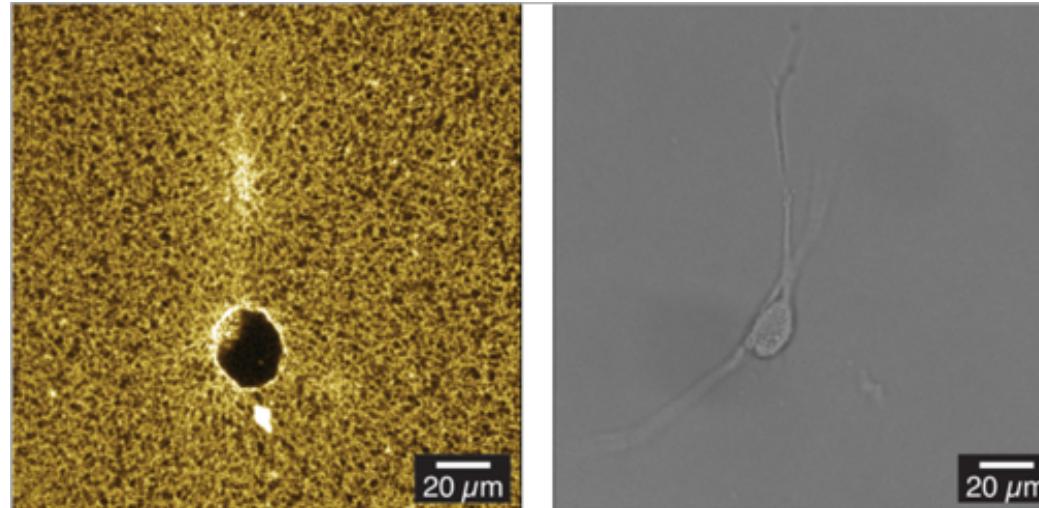


Different cell types → Different behavior

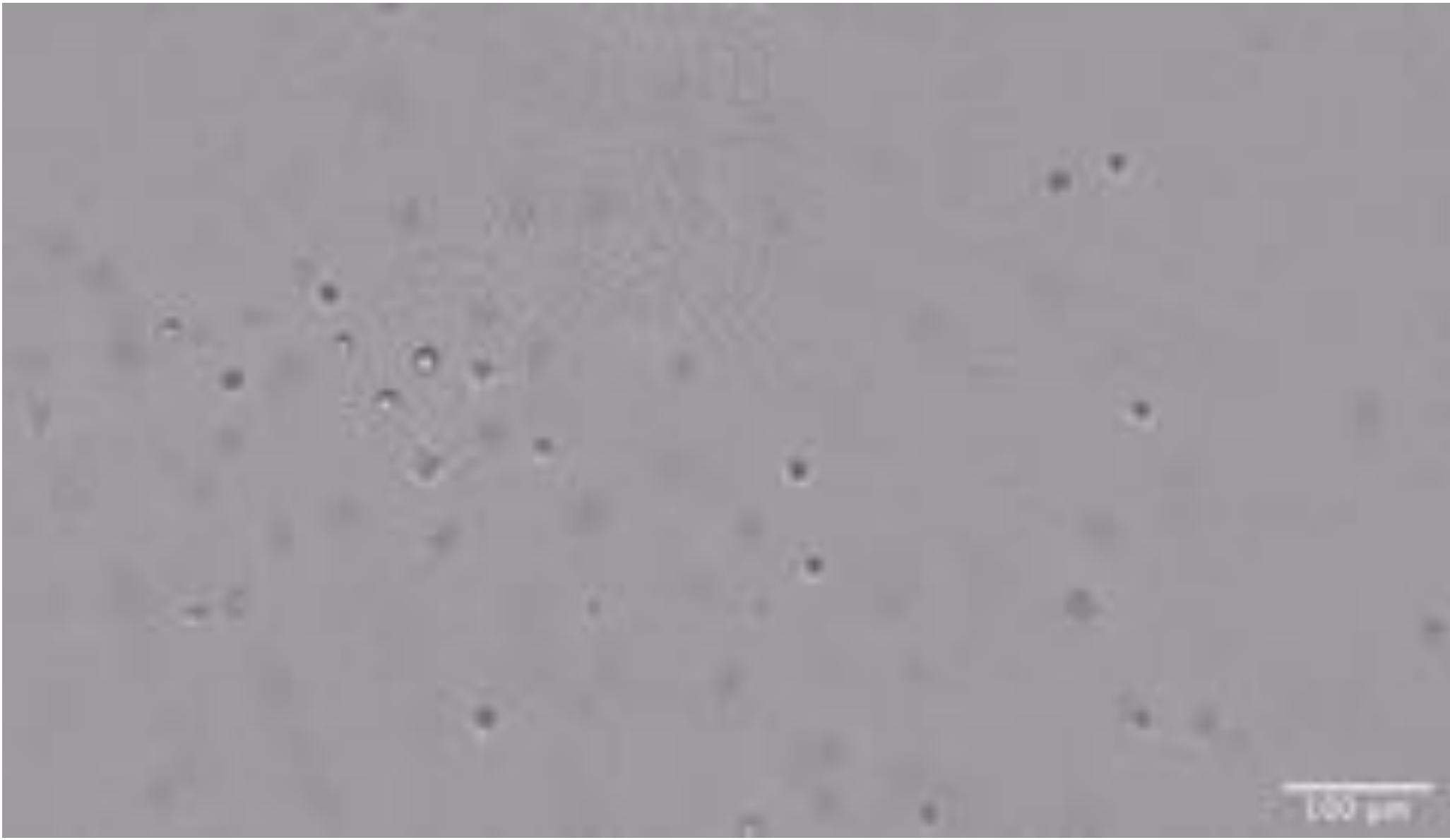


Phalloidin
DAPI

Matrix remodeling by human Adipose Stem Cells



PIC F-actin Nucleus





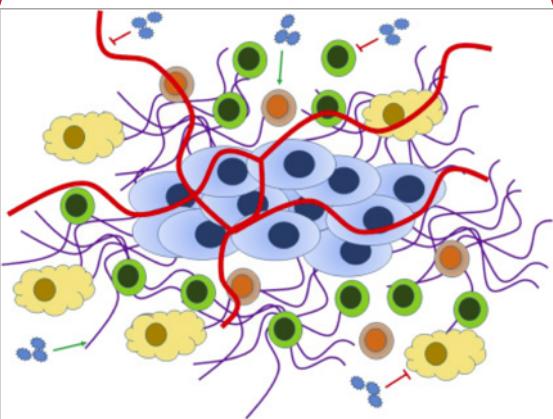
Materials in medicine

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

Susana Rocha

post-doctoral researcher
@ KU Leuven (BE)

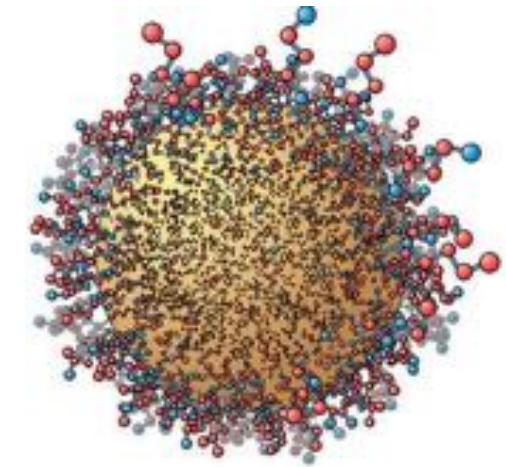
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18th June 2019



Cancer

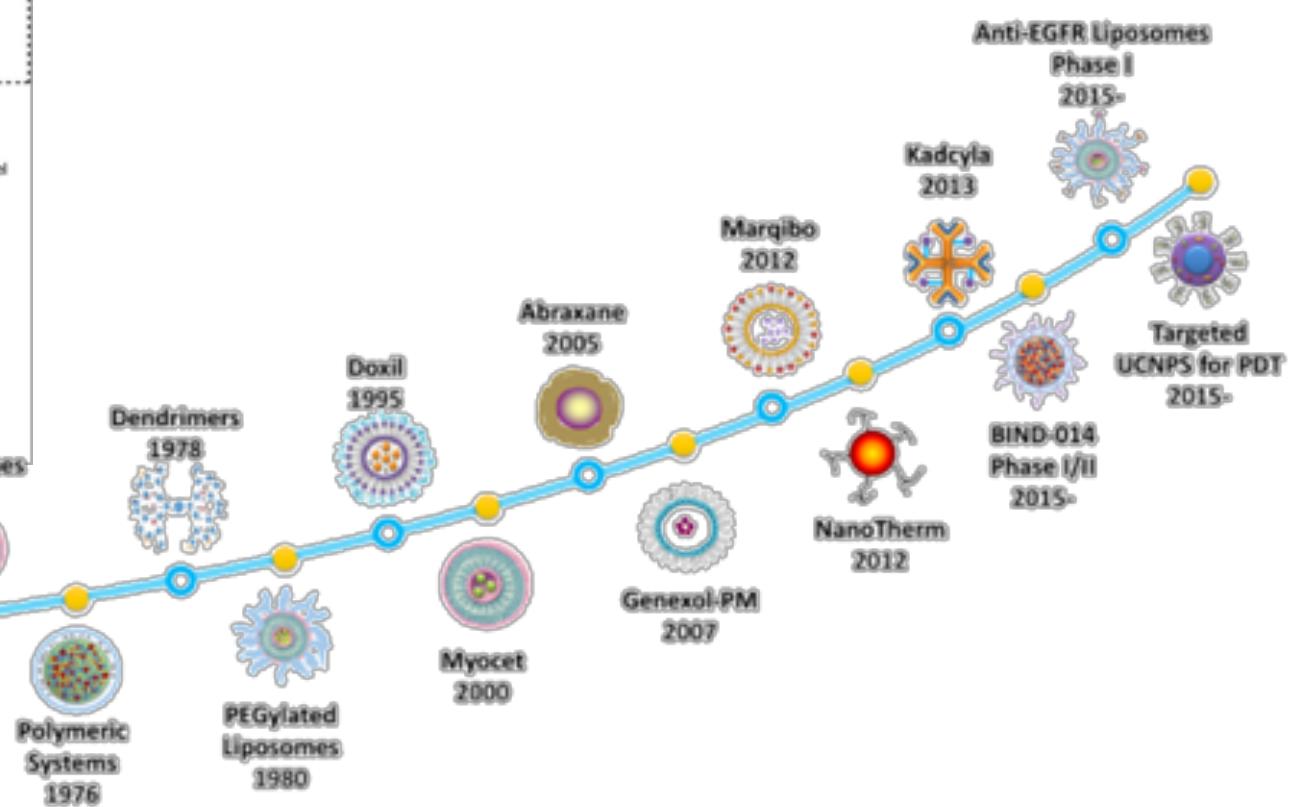
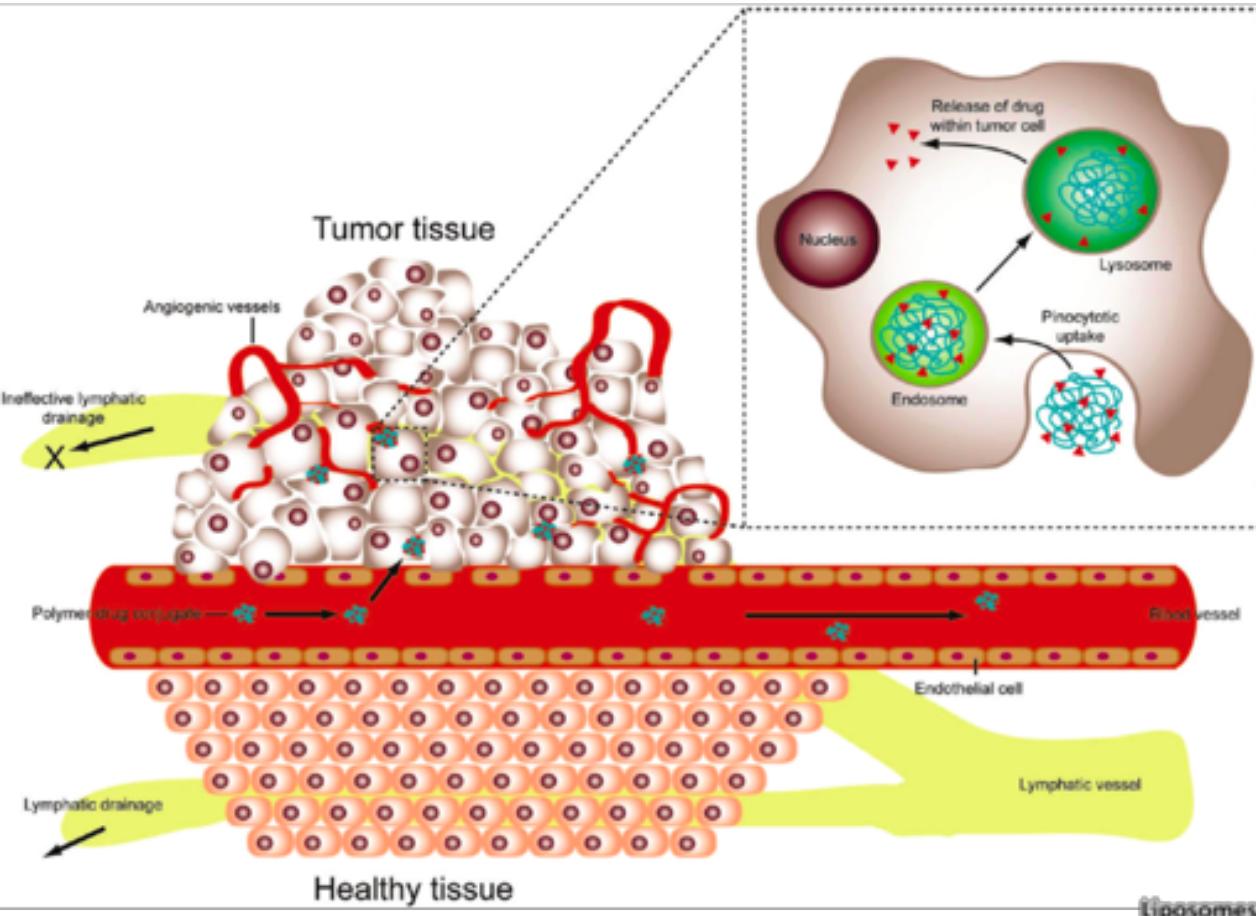


Chemotherapy

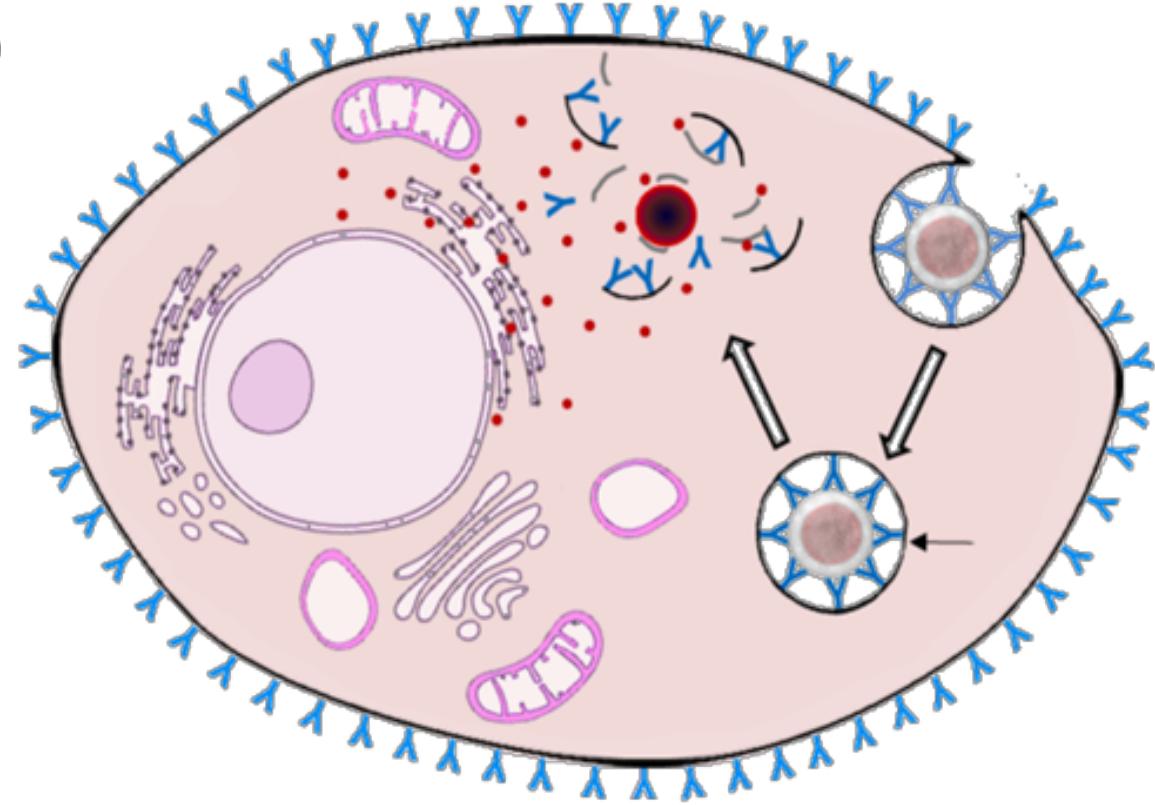


Nanocarriers

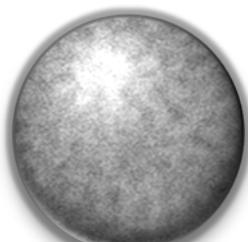
Development of nanomedicines



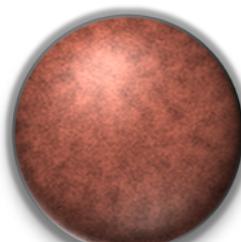
Our drug delivery system (DDS)



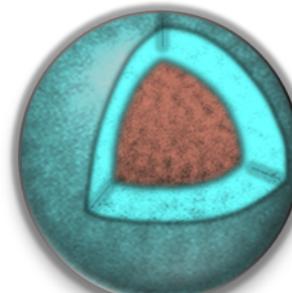
MSNP



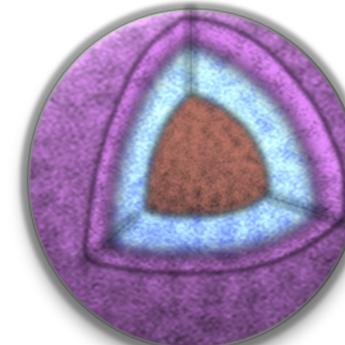
MSNP_Dox



PEI-MSNP_Dox



HAPEI-MSNP_Dox

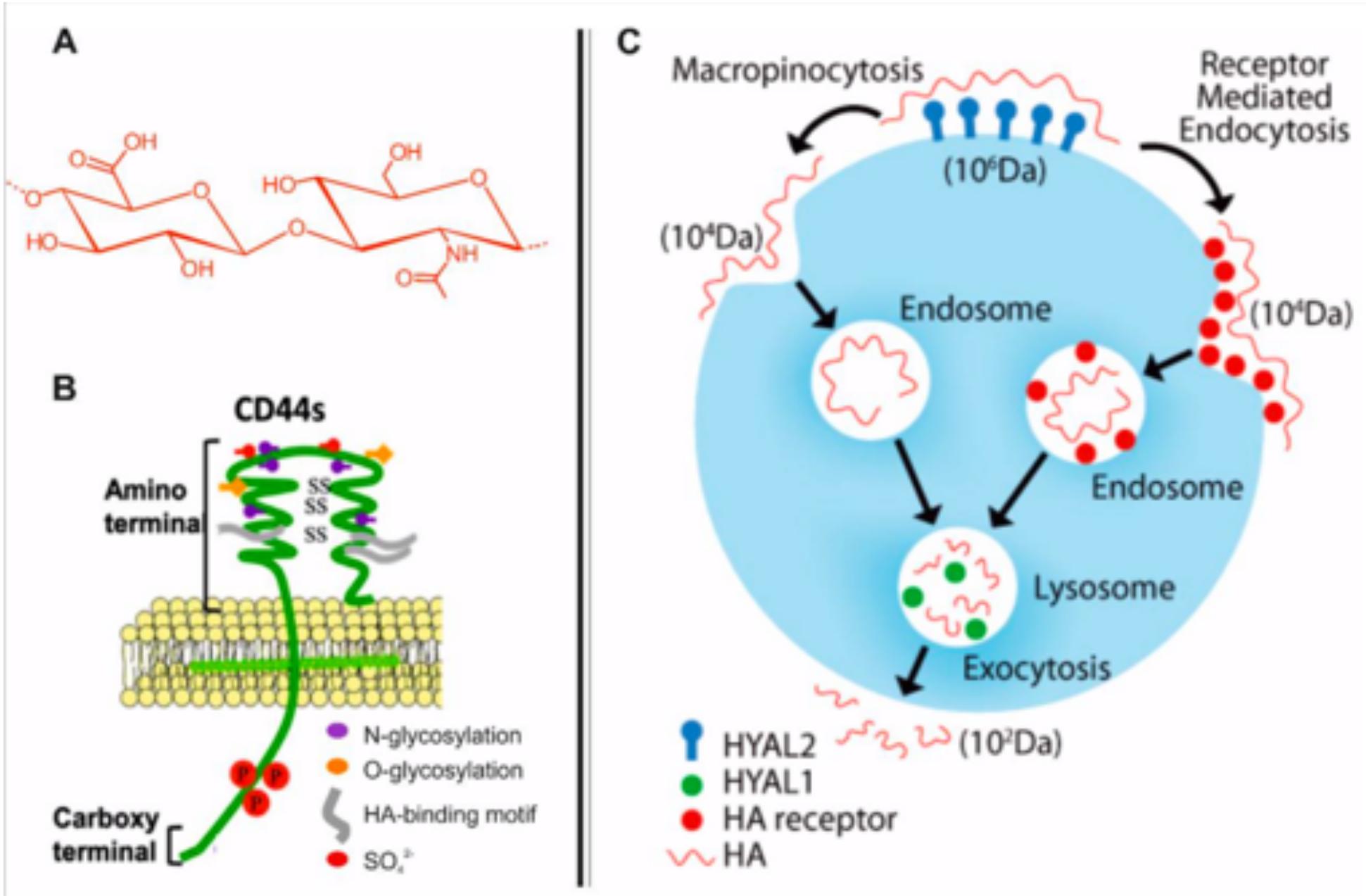


Dox

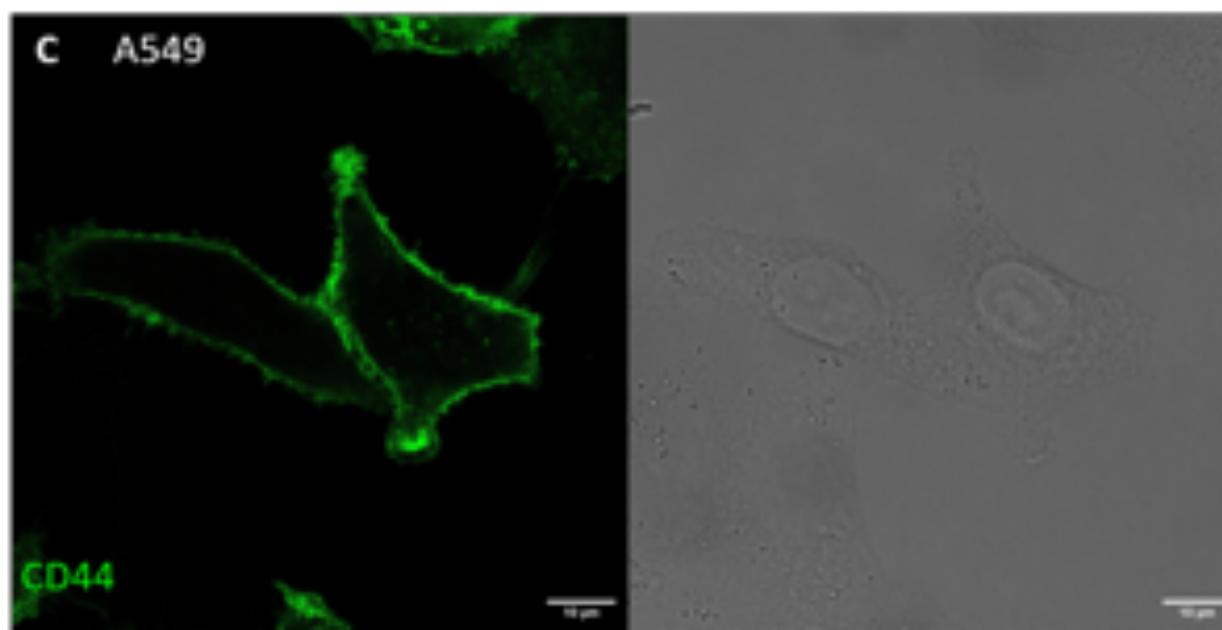
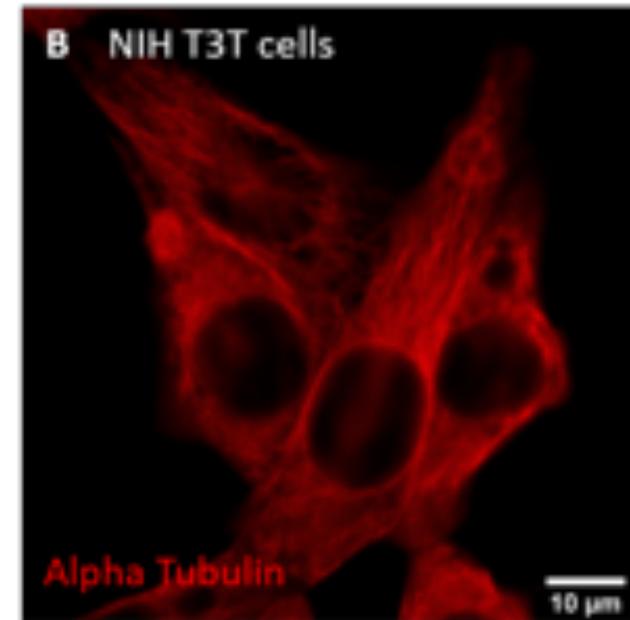
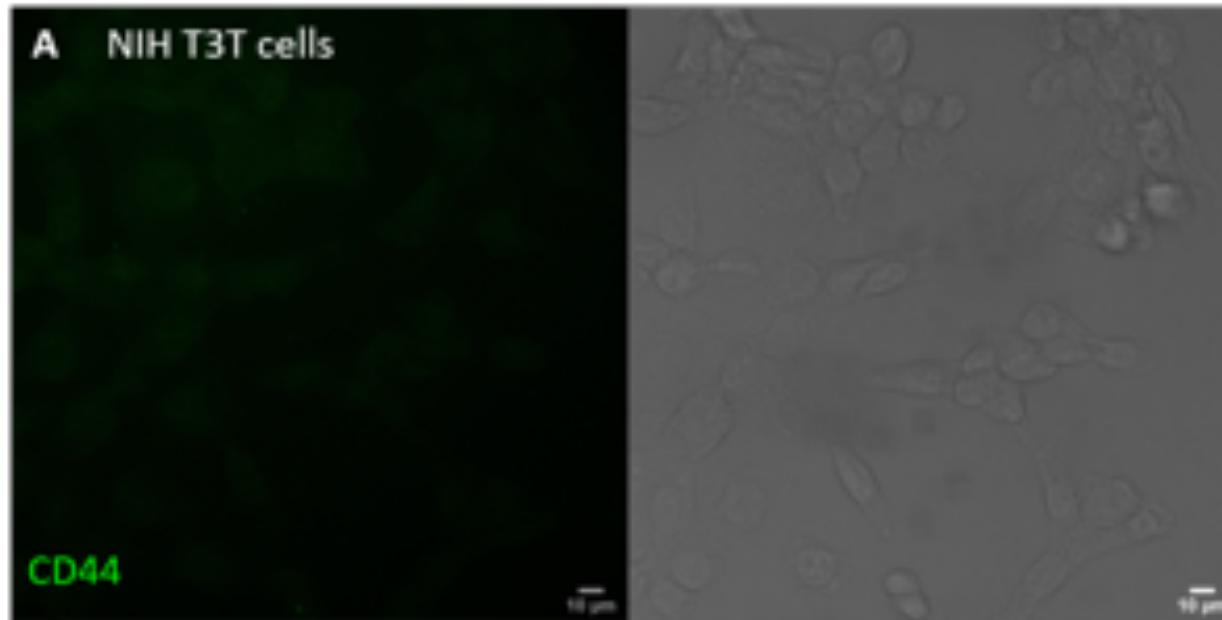
PEI

hyaluronic
acid

Targeting CD44 with HA

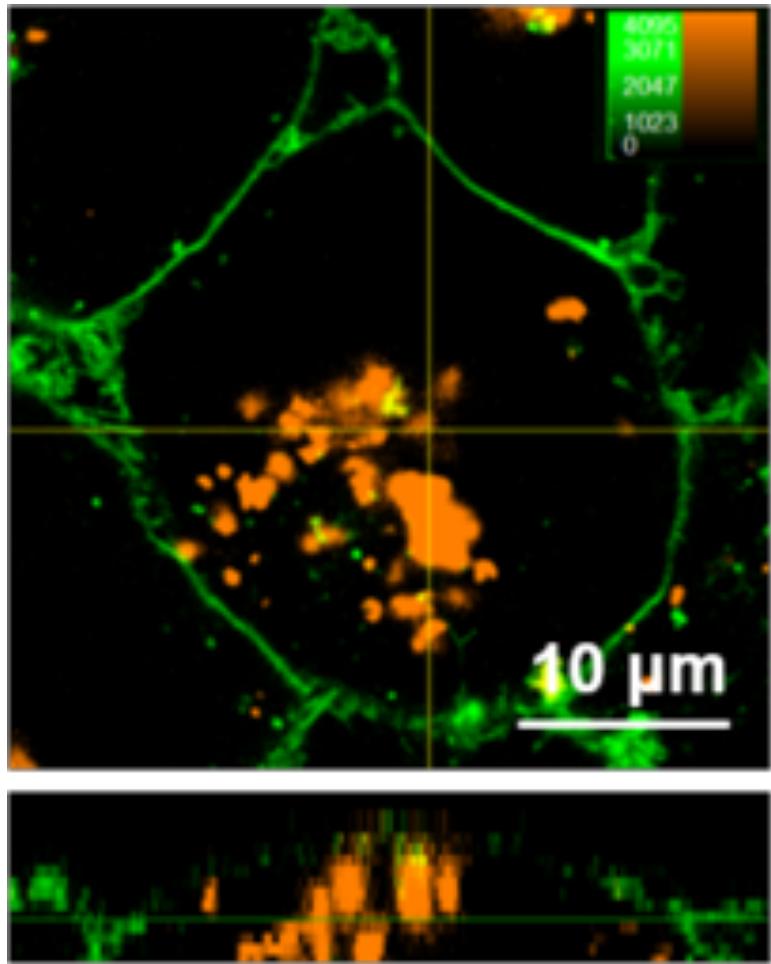


Cancer models (2D)

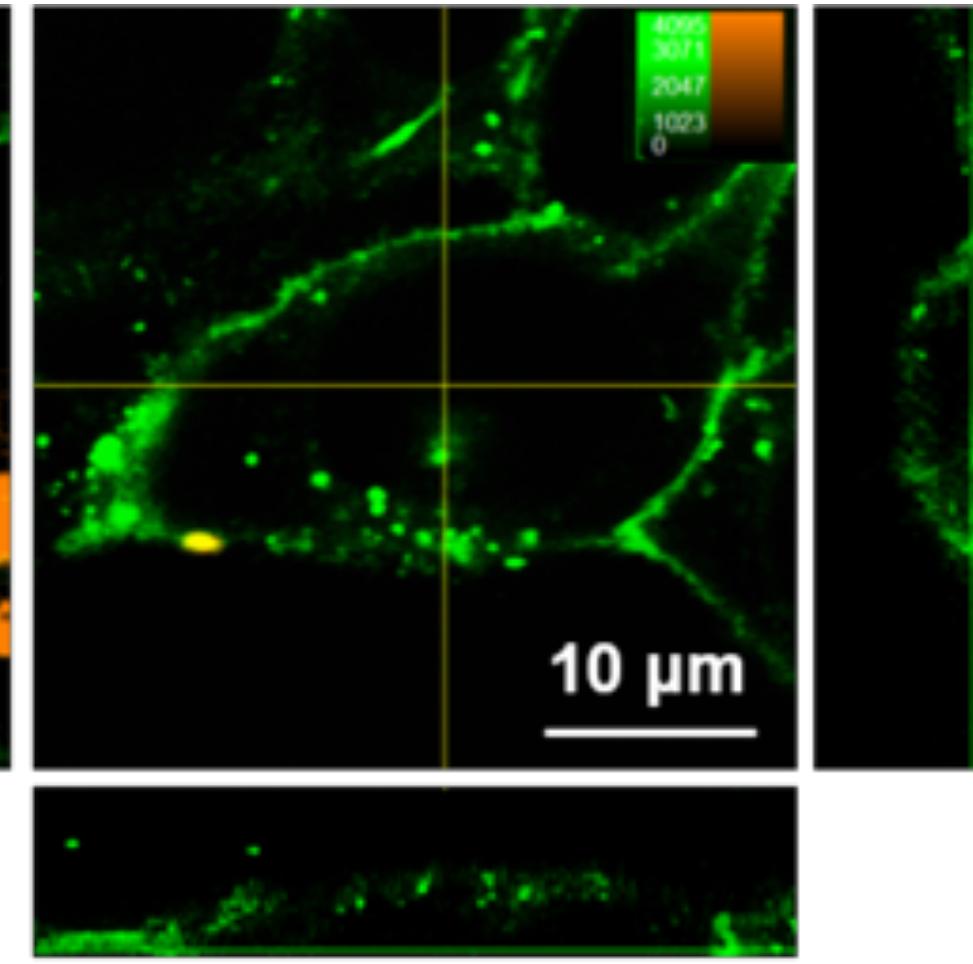


Specificity towards cancer cells

A549 (CD44+)

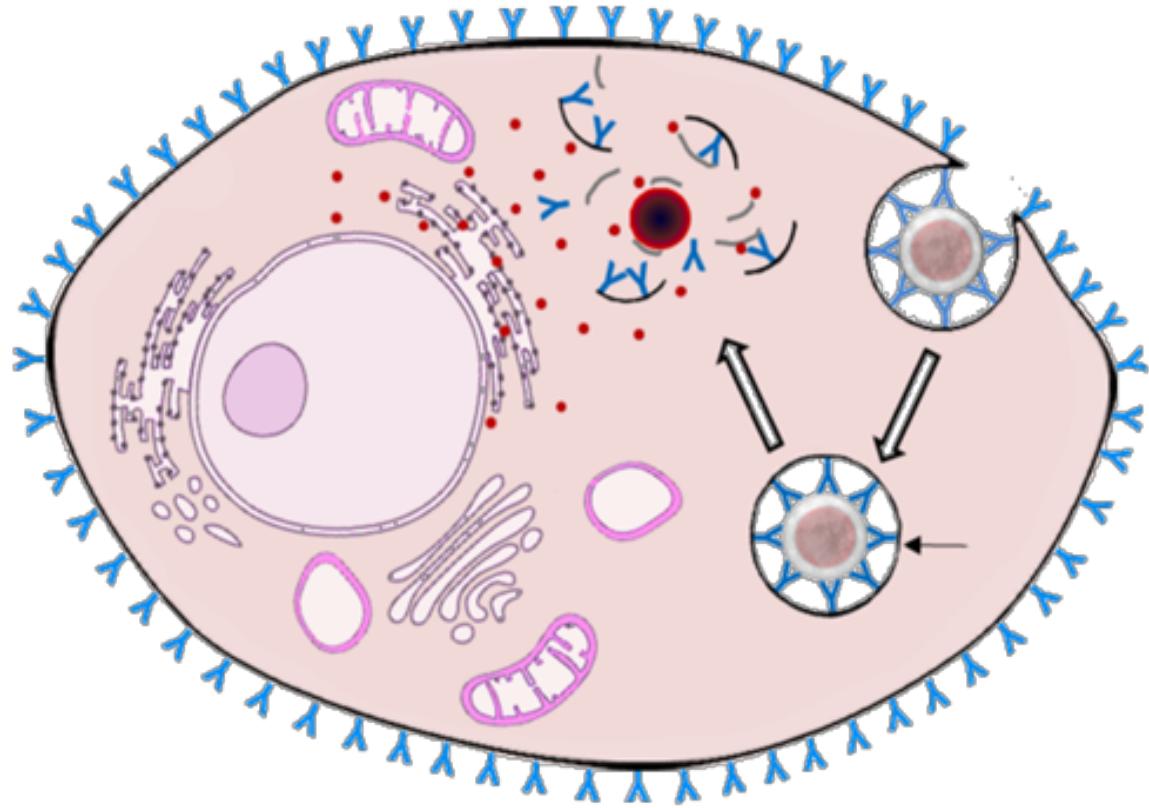


NIH 3T3 (CD44-)

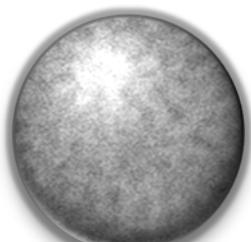


Membrane Dye (DiO)
MSNPs

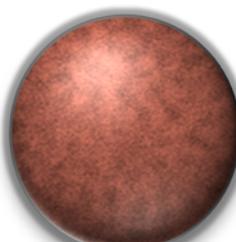
Our drug delivery system (DDS)



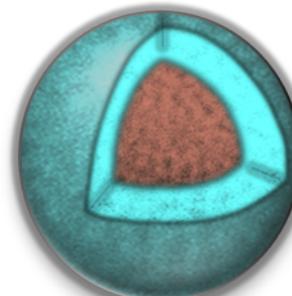
MSNP



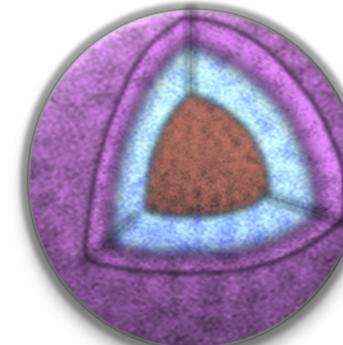
MSNP_Dox



PEI-MSNP_Dox



HAPEI-MSNP_Dox

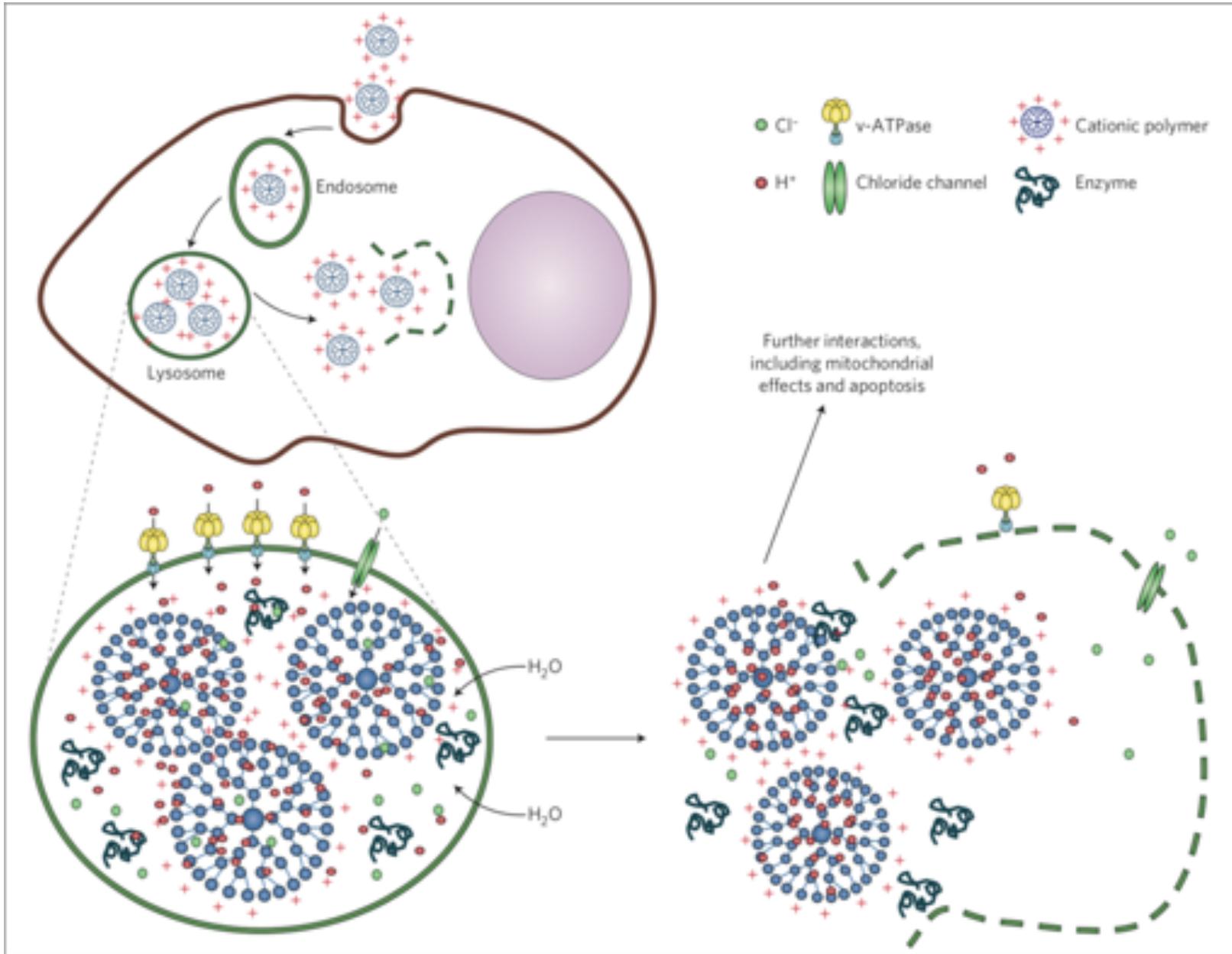


Dox

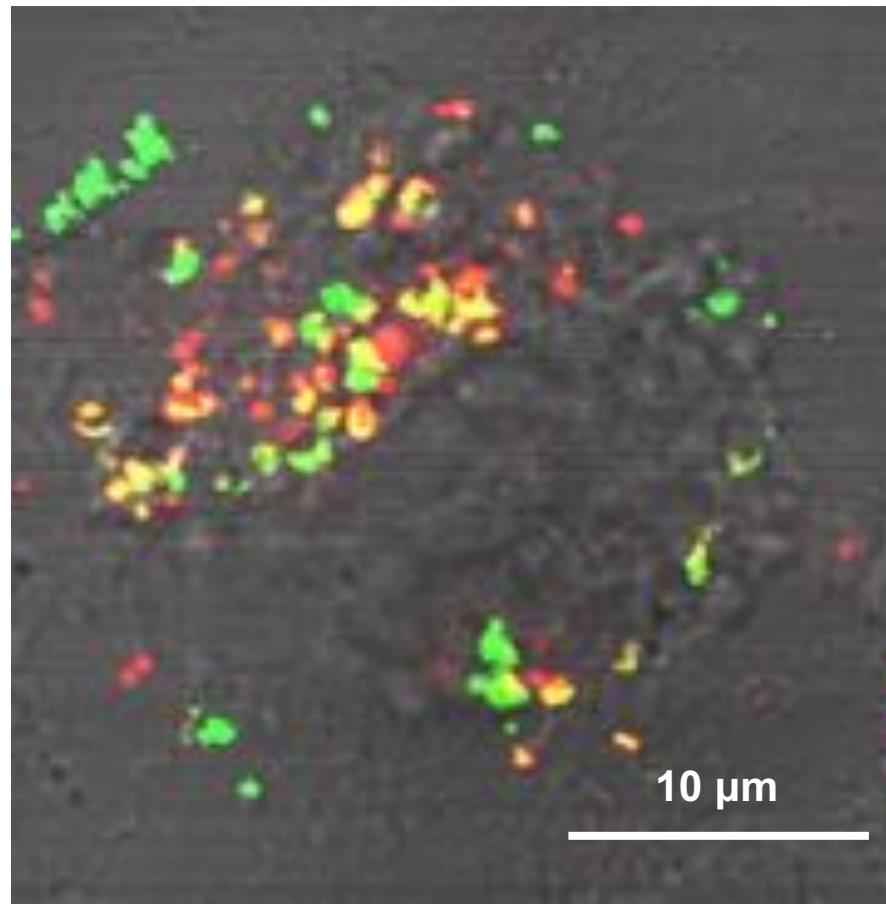
PEI

hyaluronic
acid

Endosomal escape – Proton sponge effect

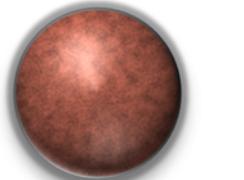


Cellular uptake and vesicle trafficking

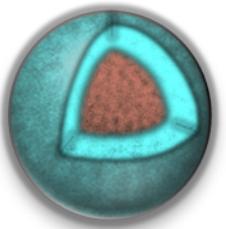
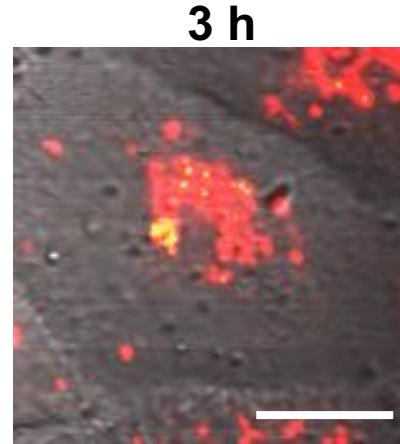


LysoTracker Red
MSNPs

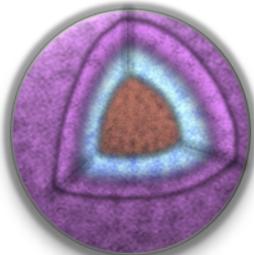
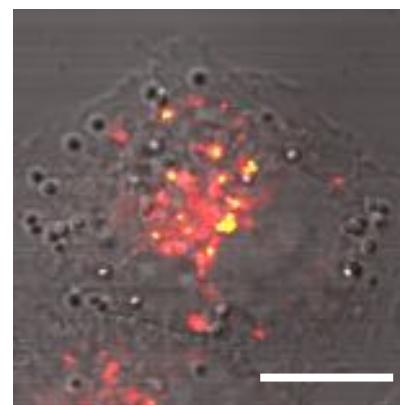
Cellular uptake and vesicle trafficking



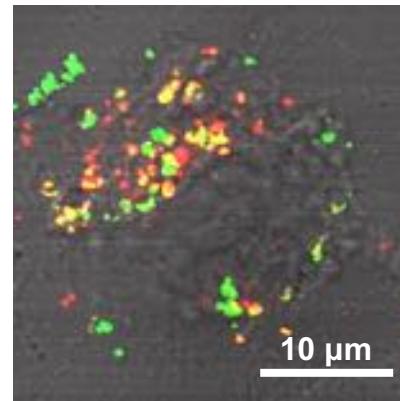
MSNPs_FITC



PEI-MSNPs_FITC

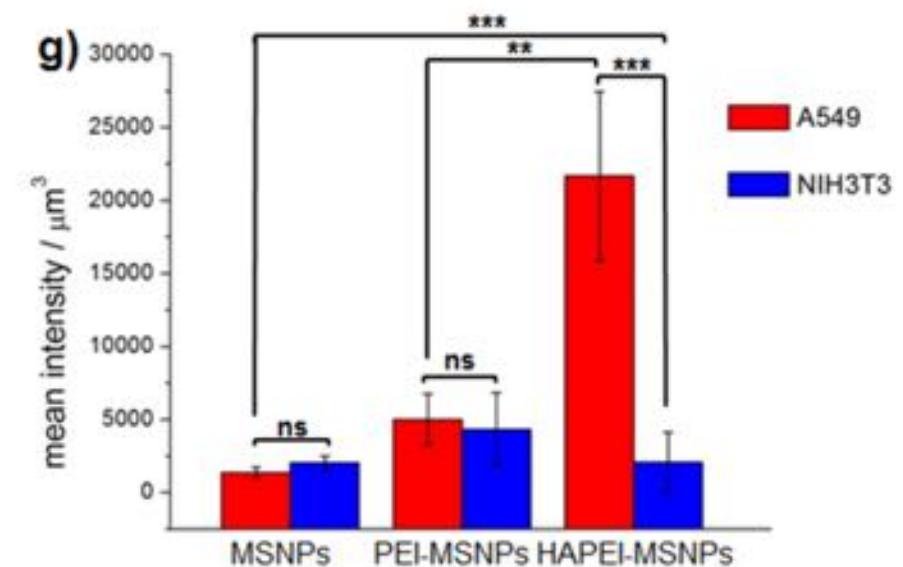
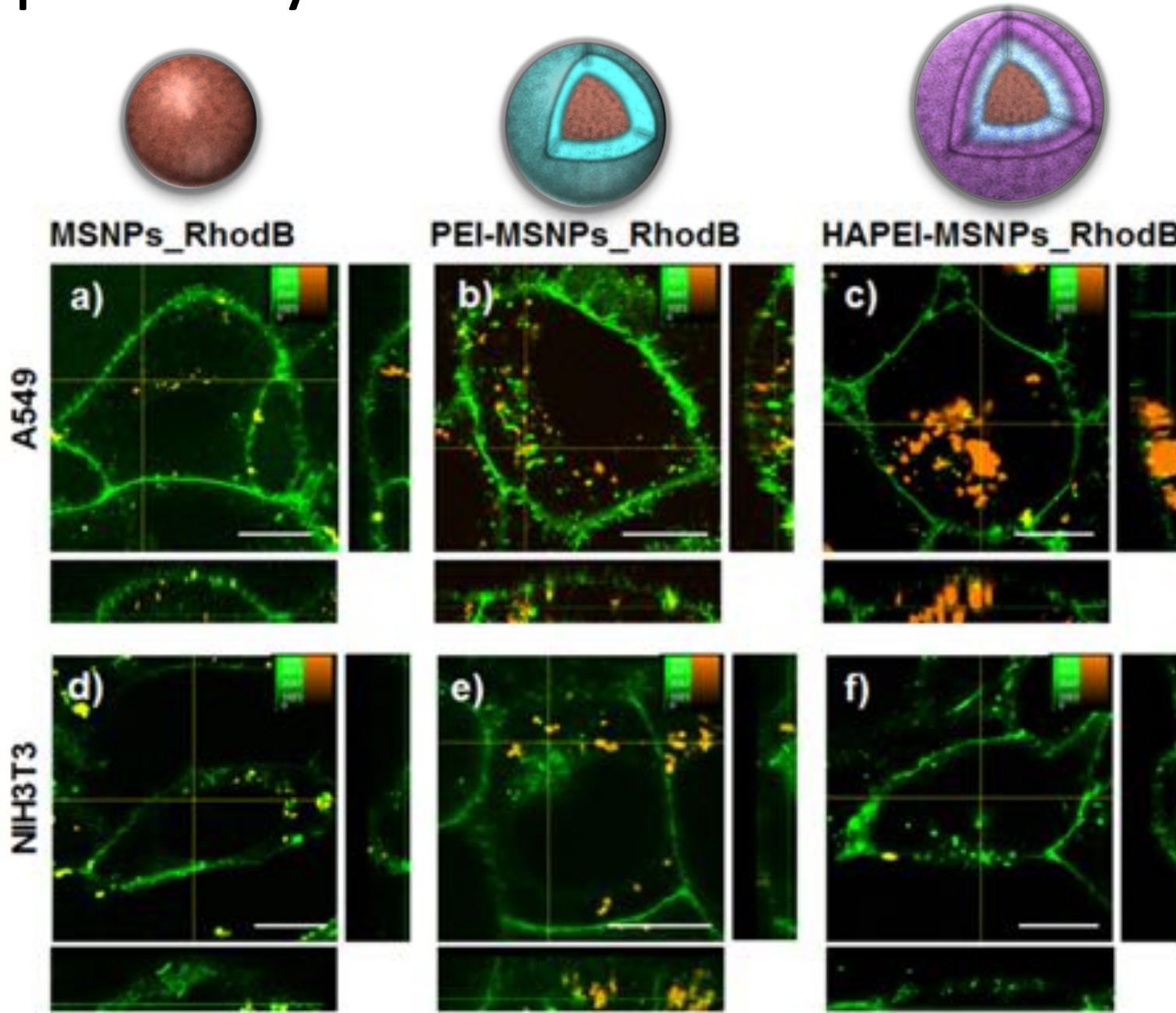


HAPEI-MSNPs_FITC



LysoTracker Red
MSNPs

Specificity to CD44+ cells

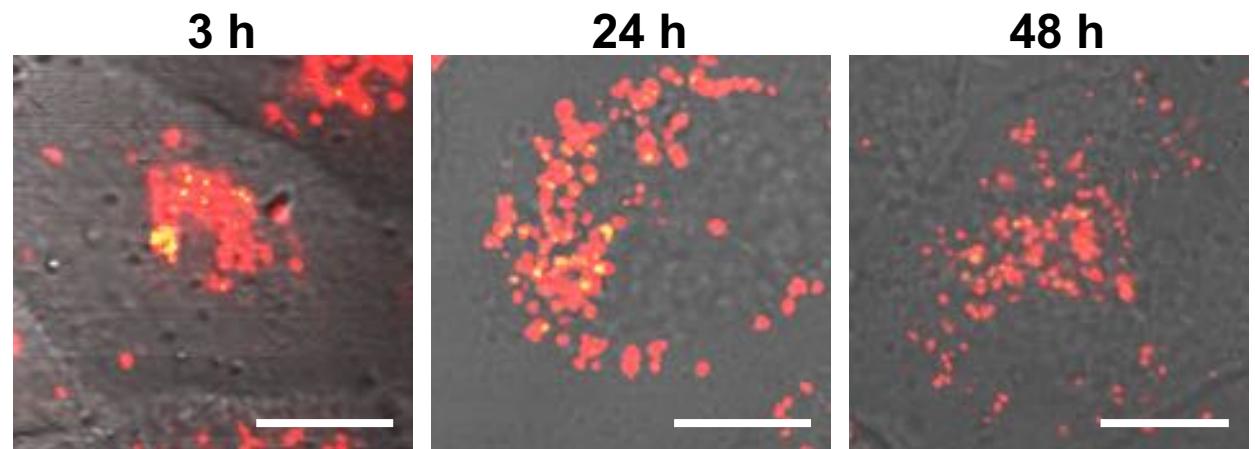


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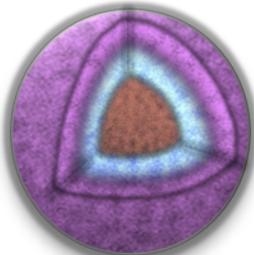
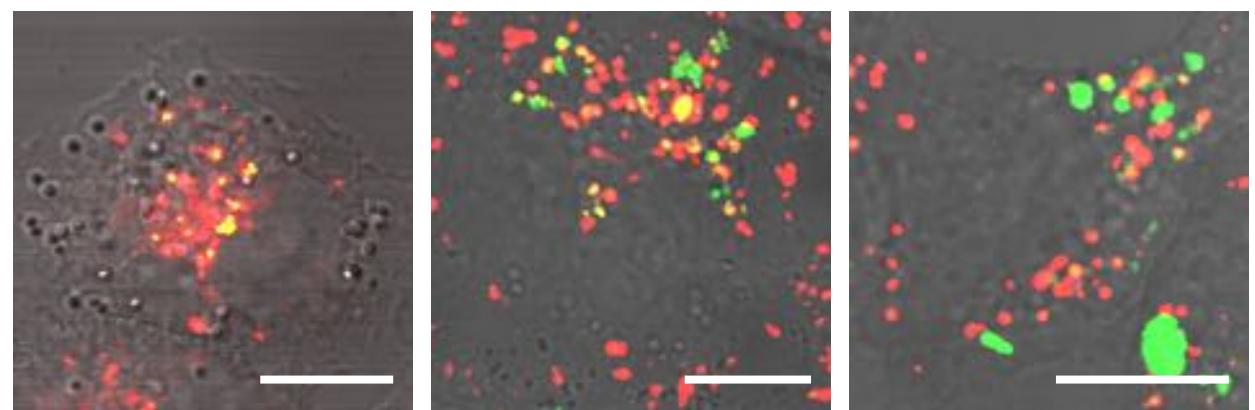
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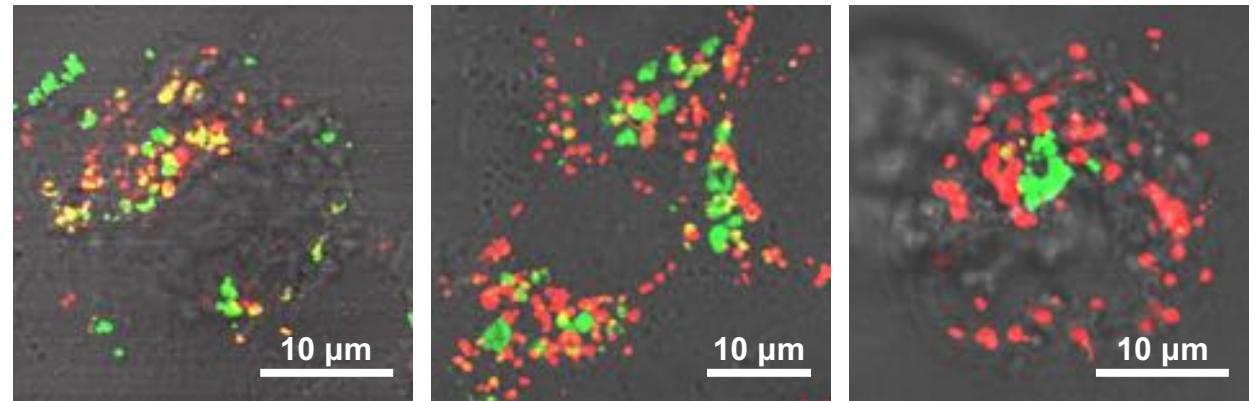
MSNPs_FITC



PEI-MSNPs_FITC



HAPEI-MSNPs_FITC

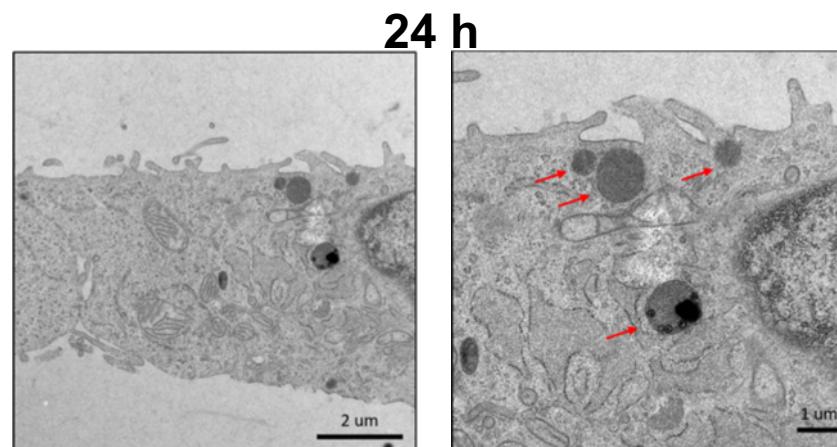


LysoTracker Red
MSNPs

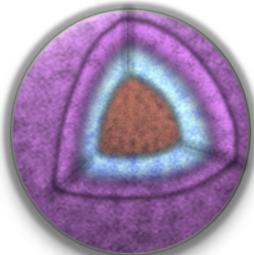
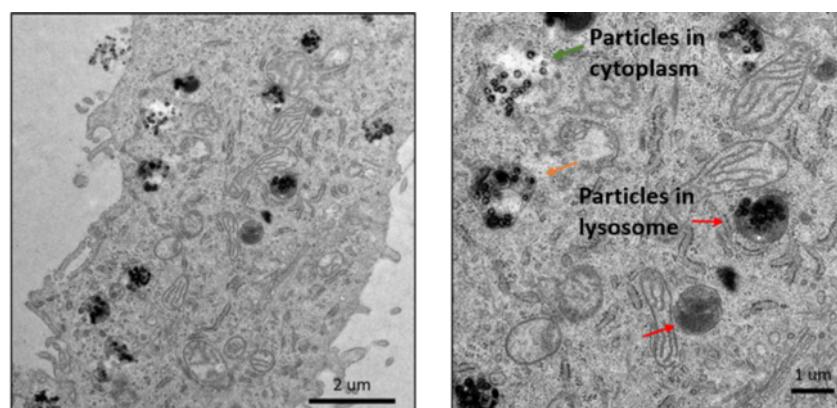
Cellular uptake and vesicle trafficking



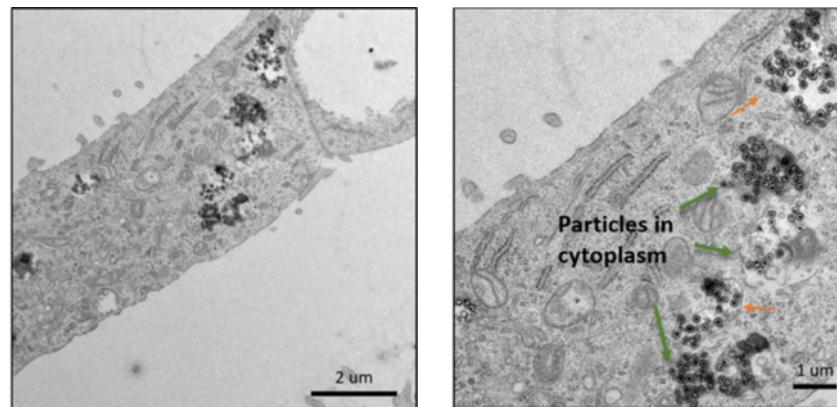
Au@MSNPs



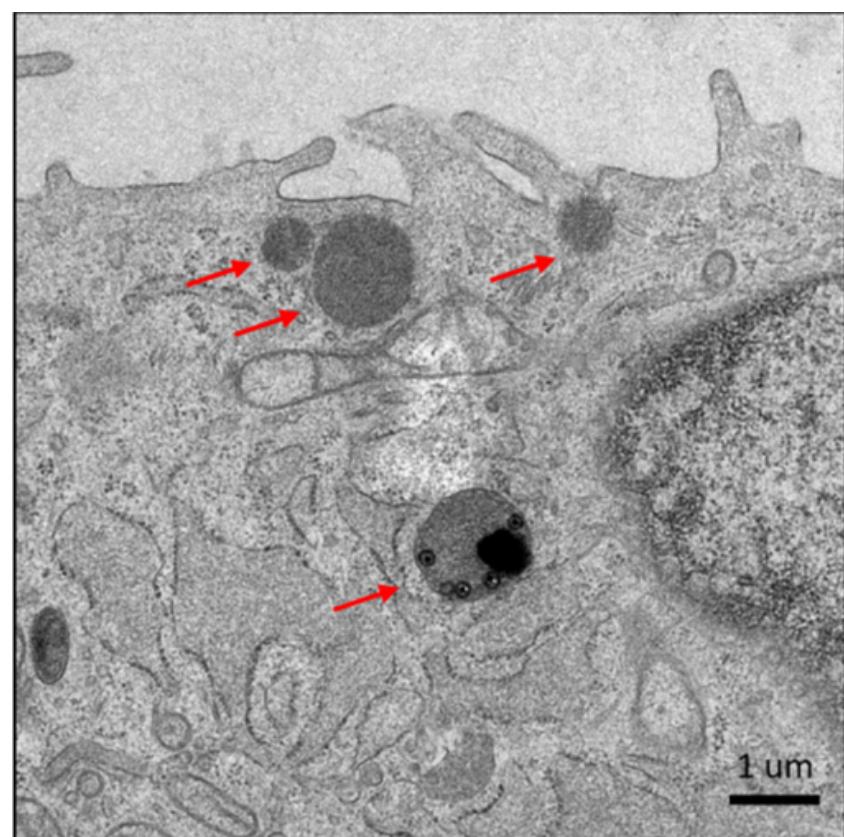
PEI-Au@MSNPs



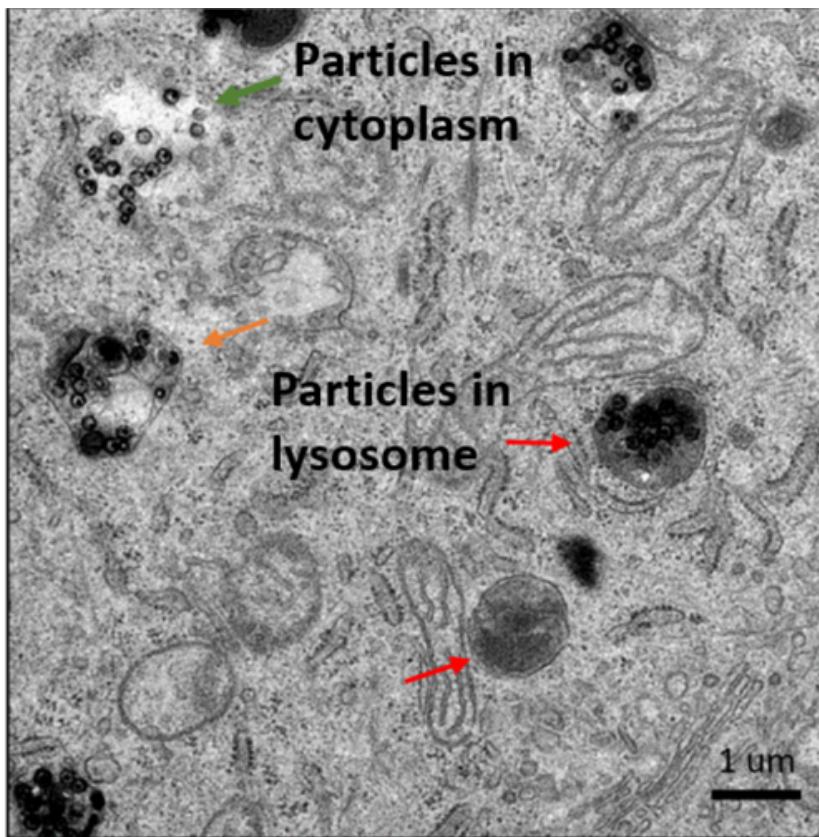
HAPEI-Au@MSNPs



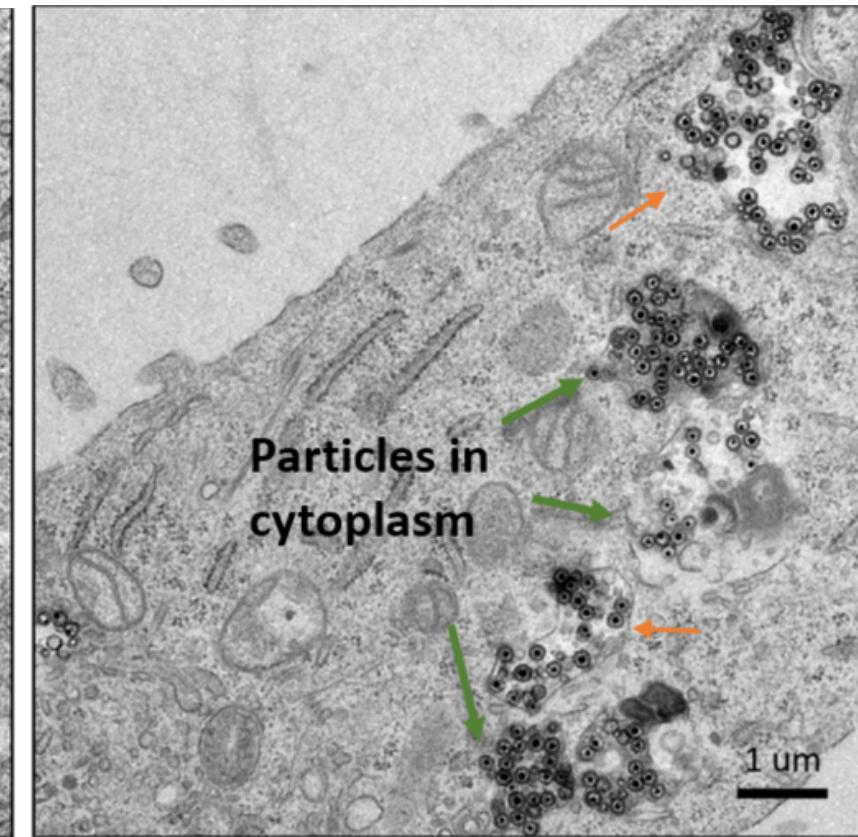
Cellular uptake and vesicle trafficking



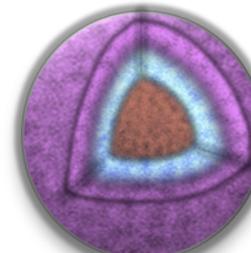
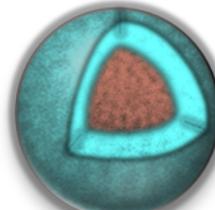
Au@MSNPs



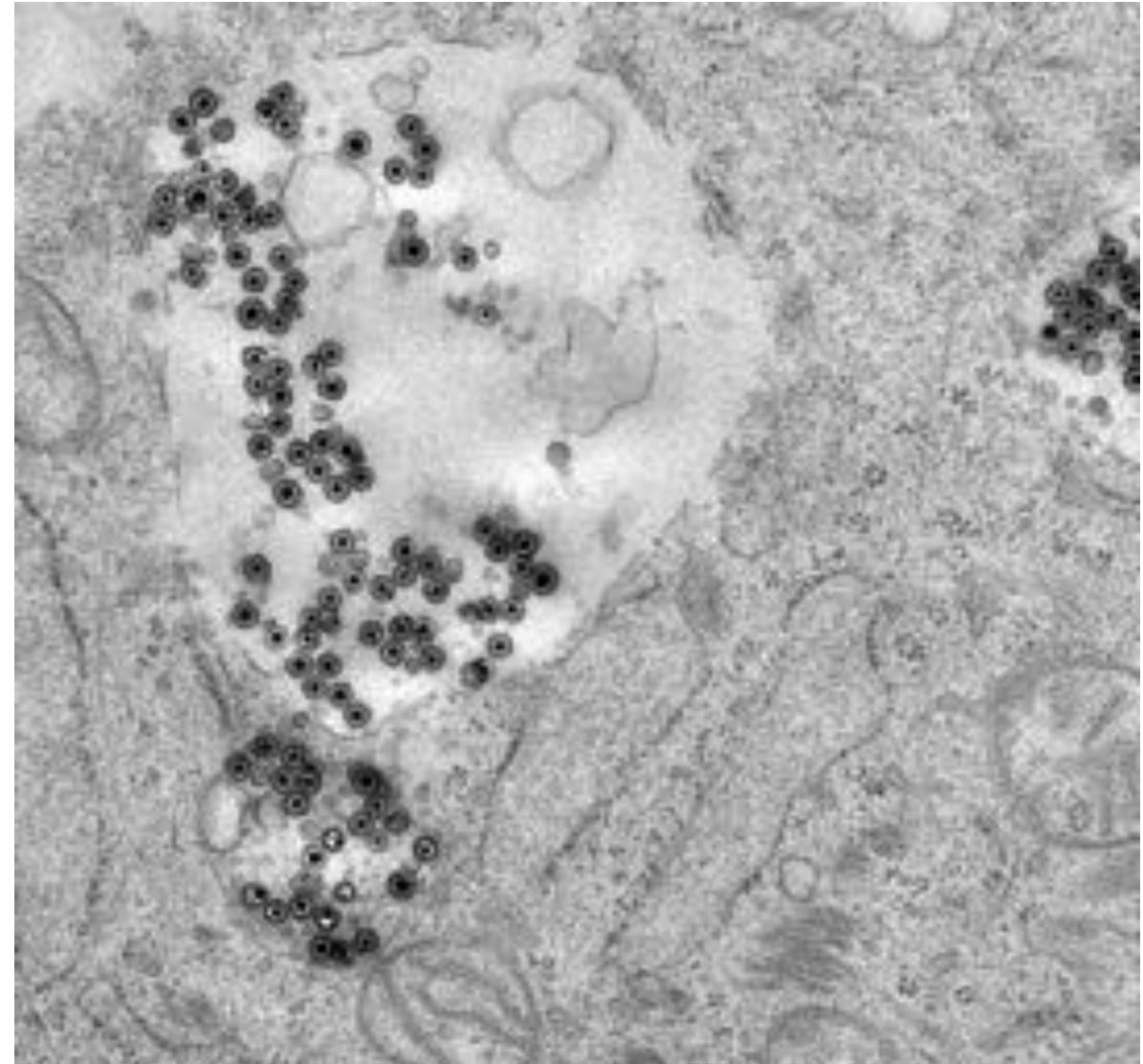
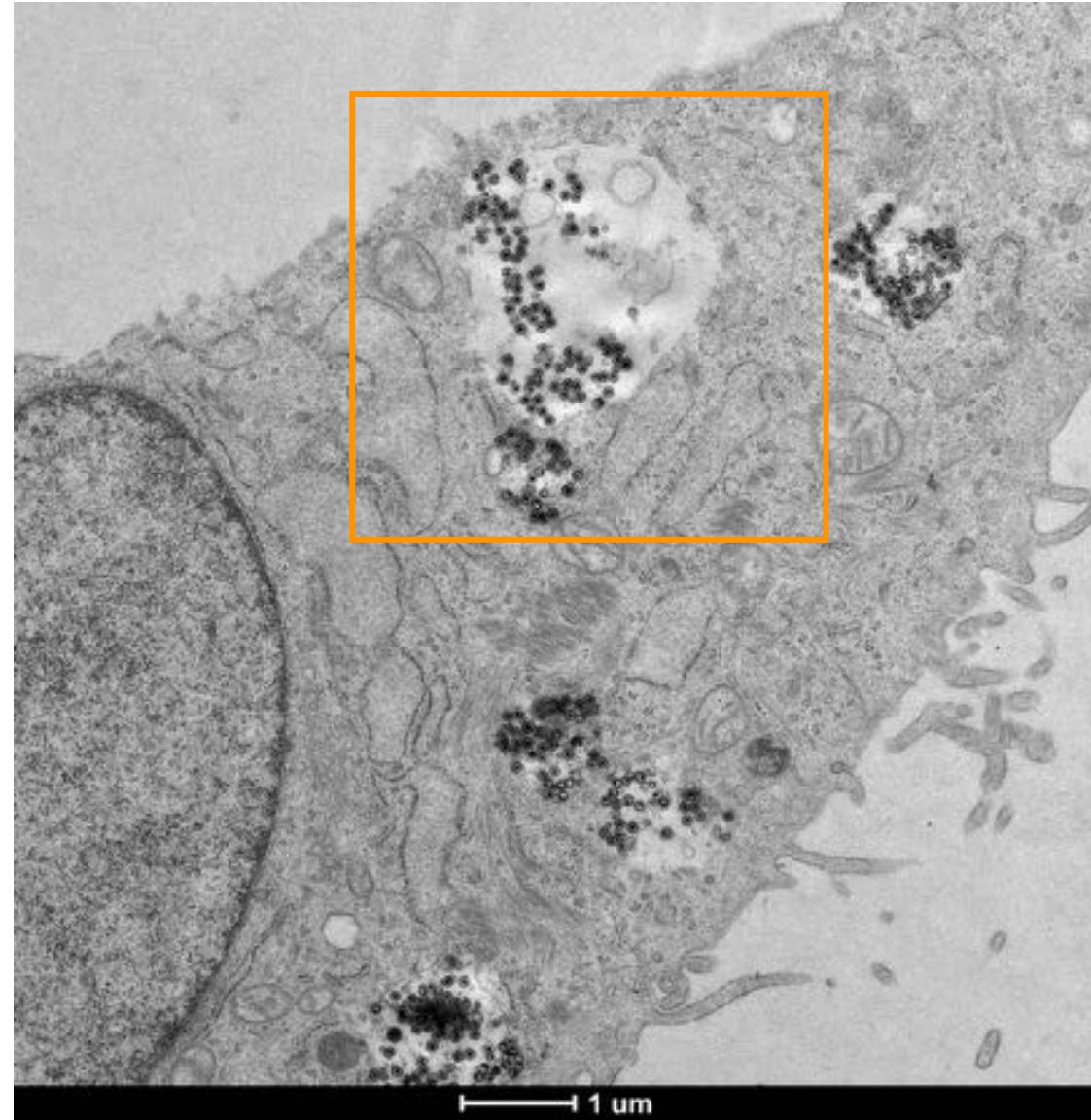
PEI-Au@MSNPs



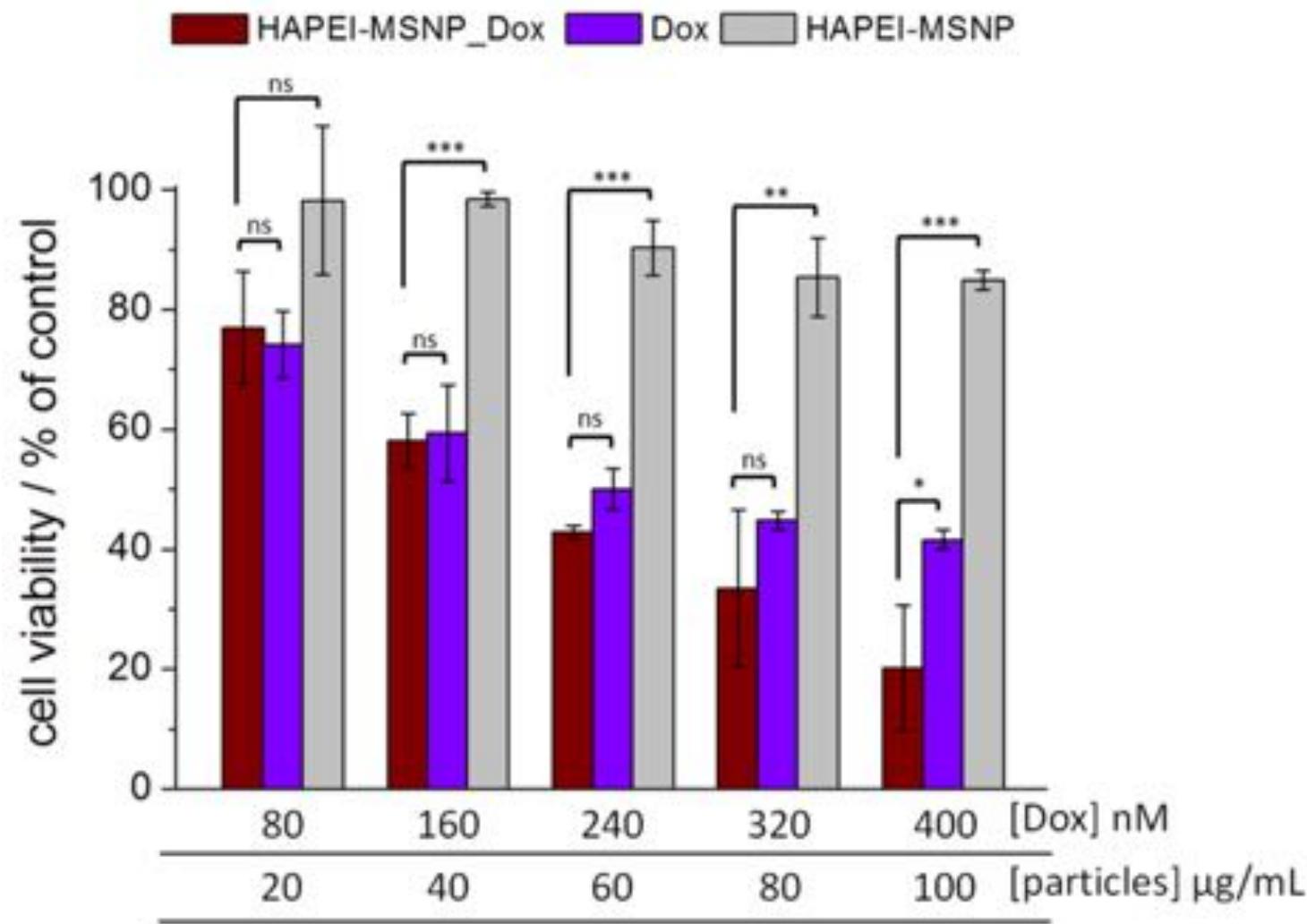
HAPEI-Au@MSNPs

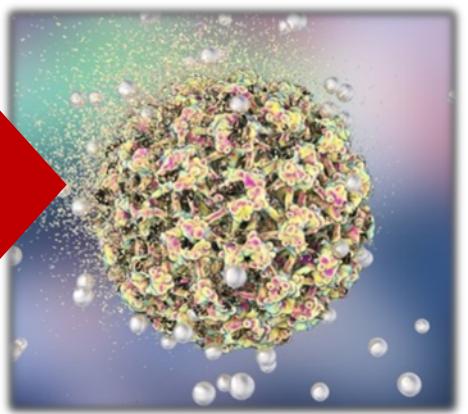


Endosomal rupture?!?

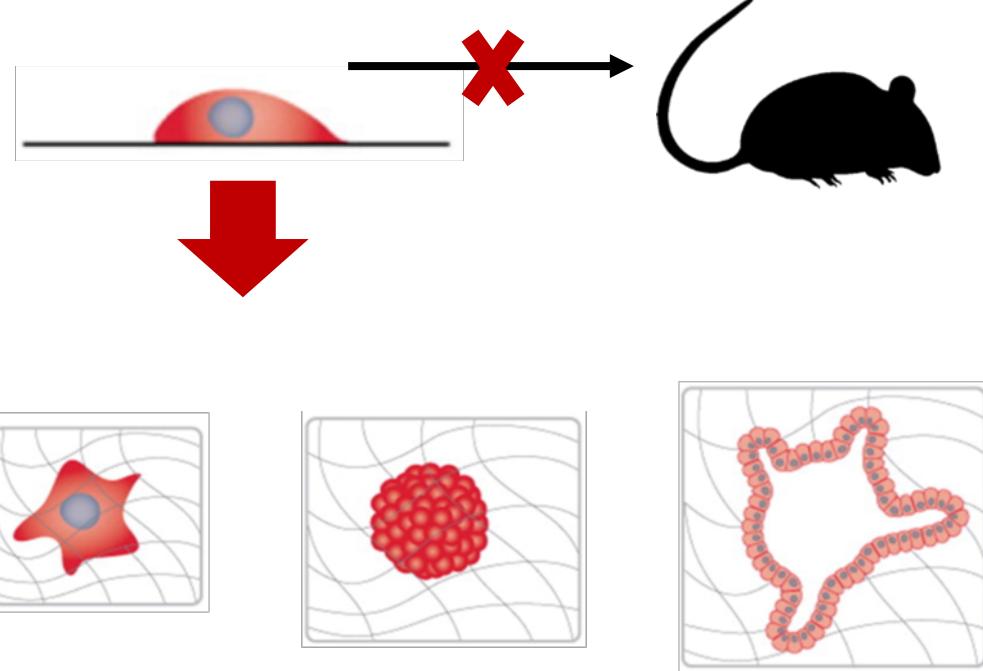


Anticancer efficiency of Dox-loaded particles



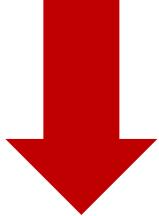


The (nano)medicine



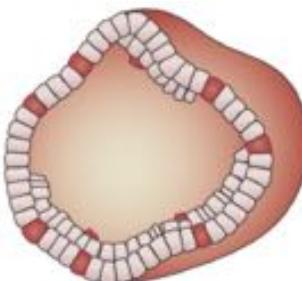
The model

What is the
intercellular route of
the nanoparticles?



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

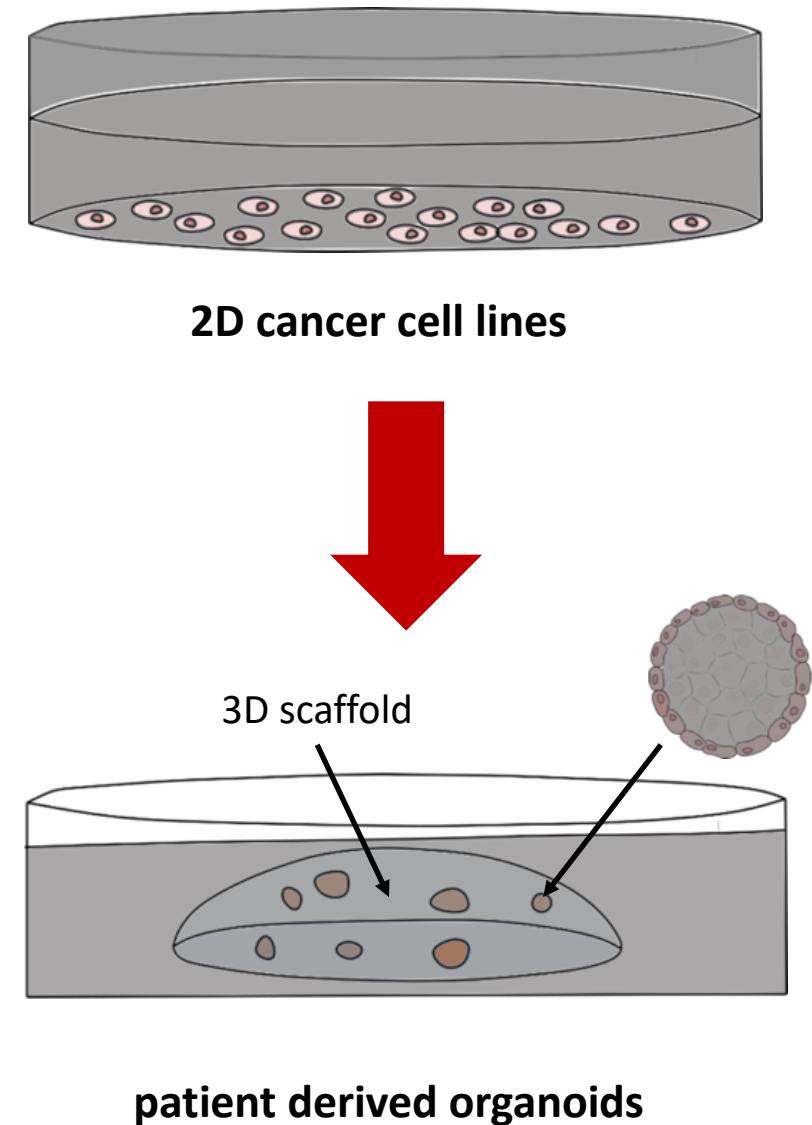
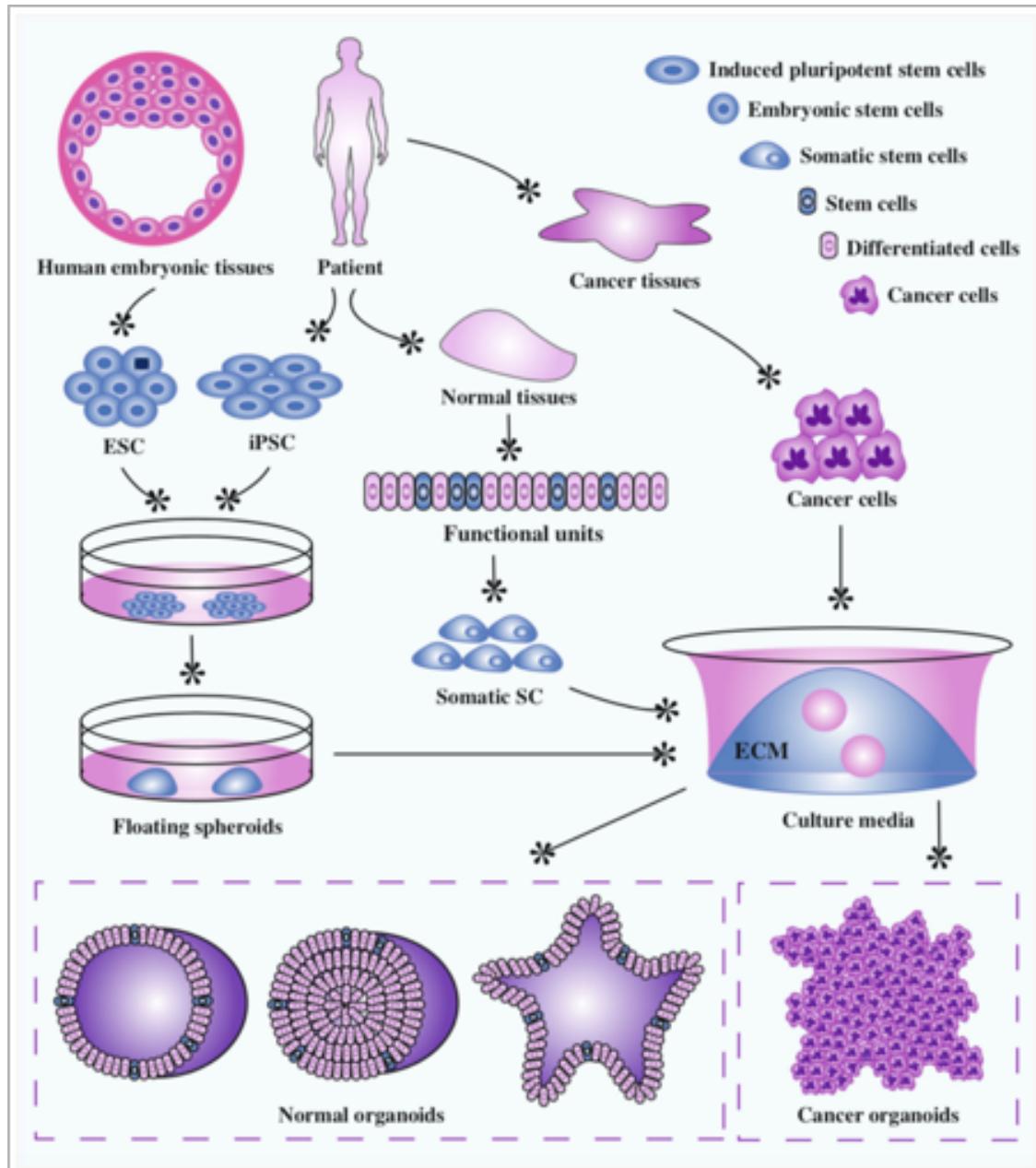
Diffusion



Transcytosis
or/and para
cellular
transport?

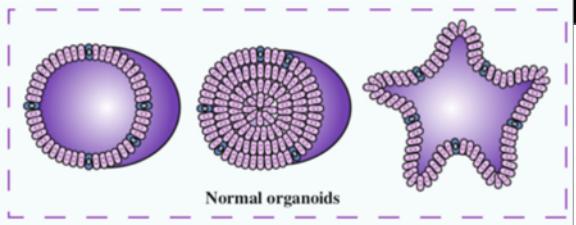
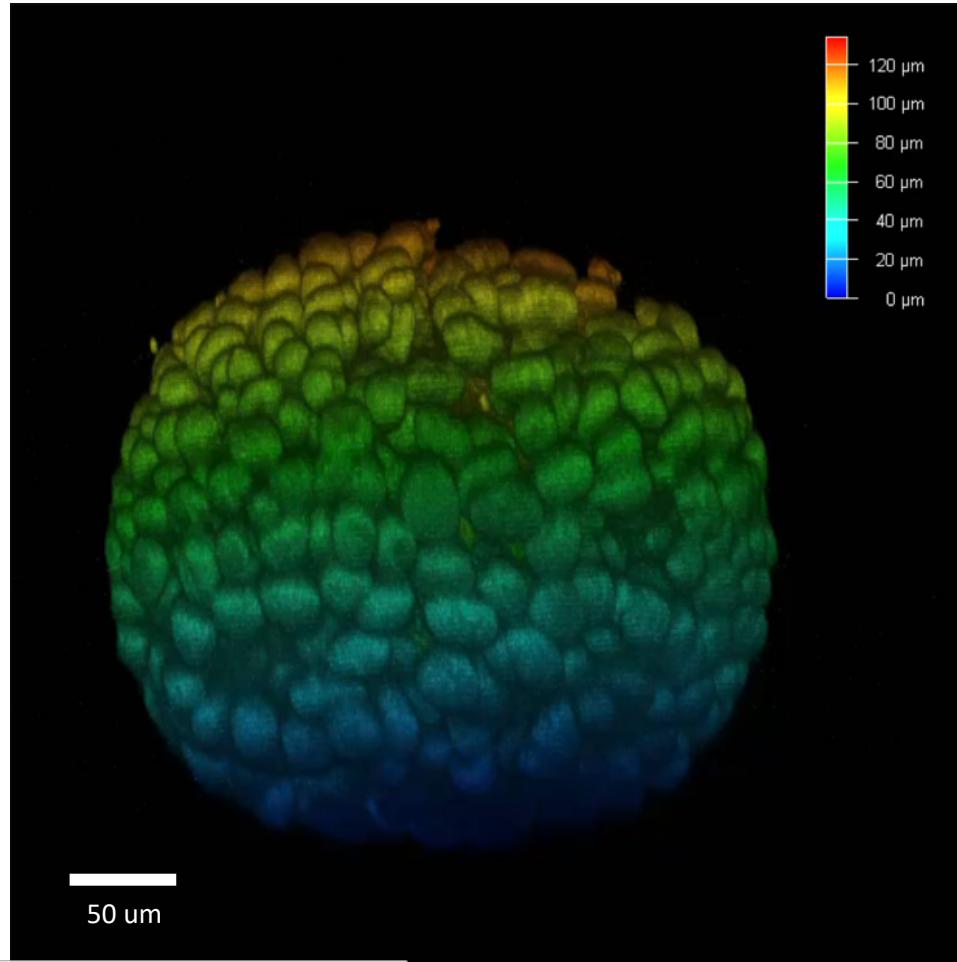
Permeability

Organoids as realistic *in vitro* cancer models

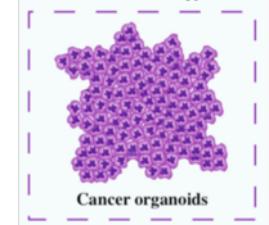
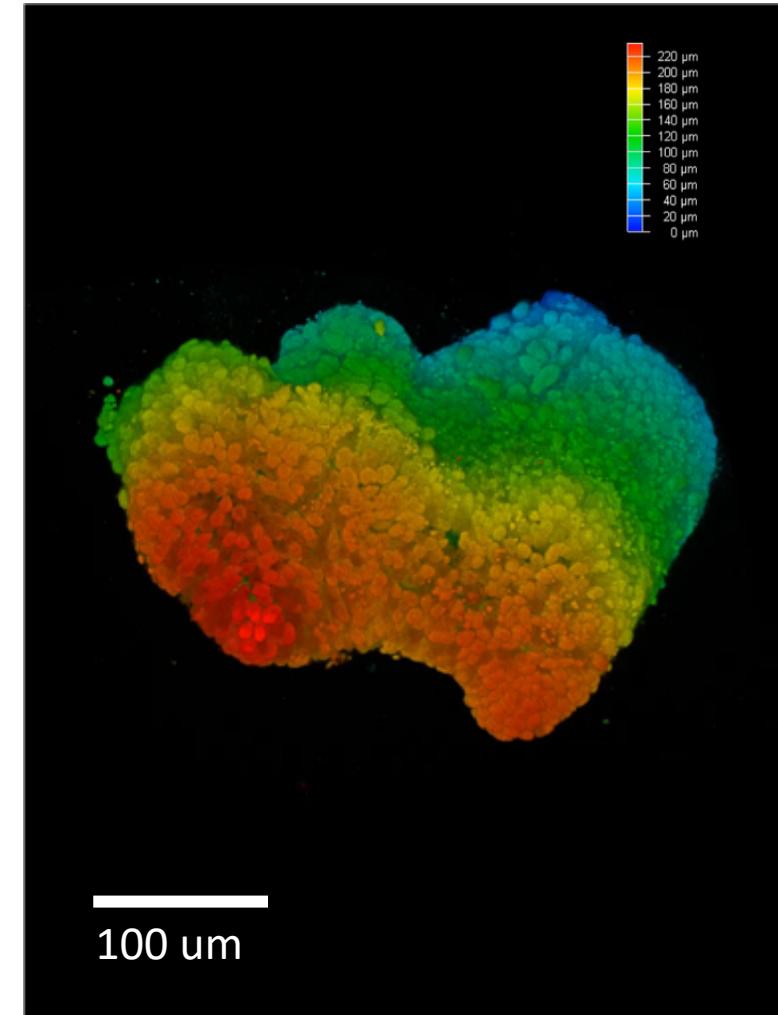


Organoids as realistic *in vitro* cancer models

Healthy endometrial organoid

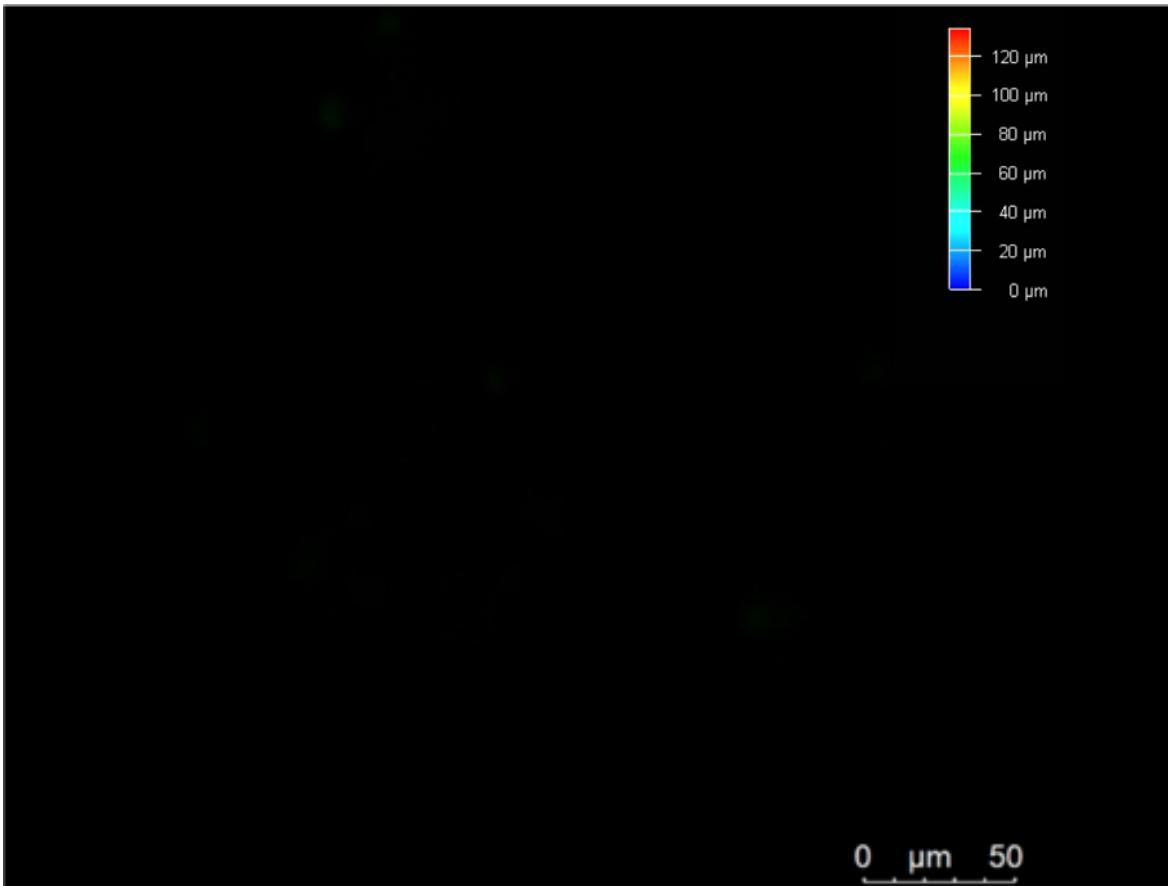


endometrial cancer organoid

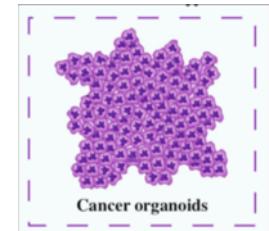
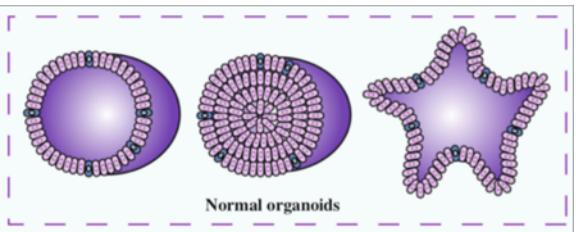
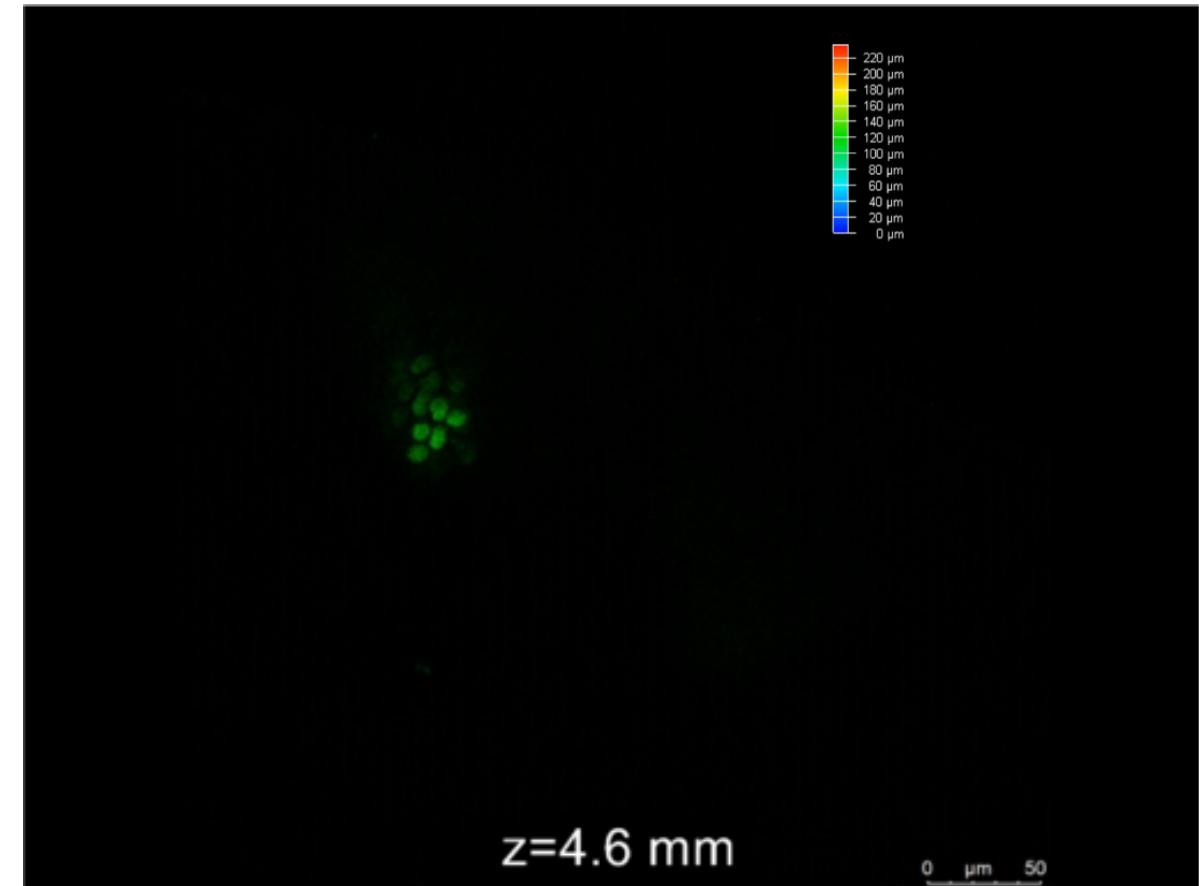


Organoids as realistic *in vitro* cancer models

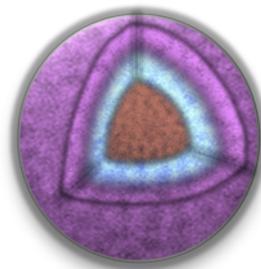
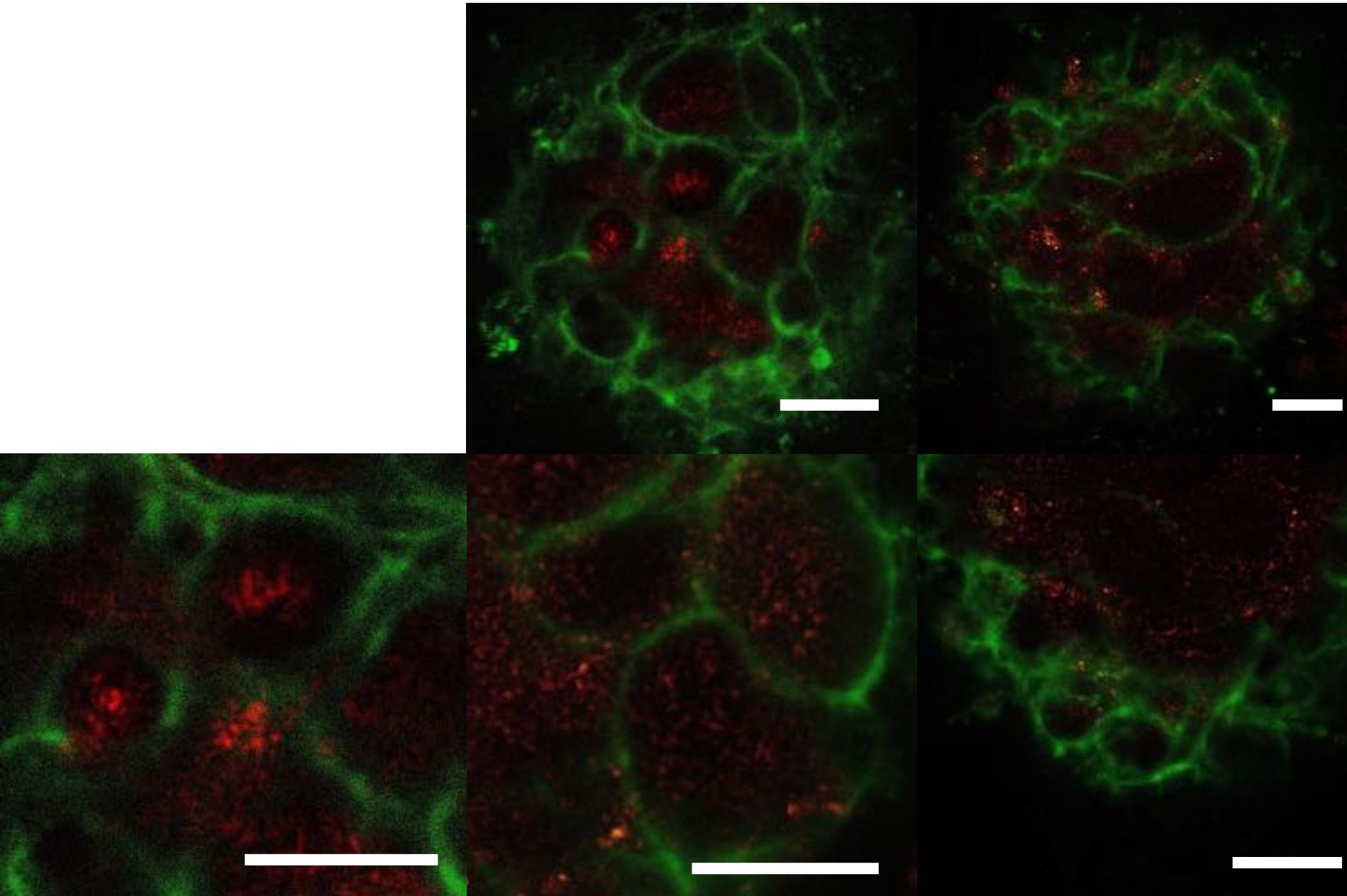
Healthy endometrial organoid



endometrial cancer organoid



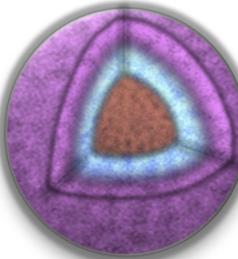
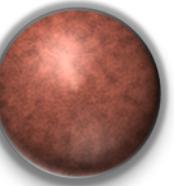
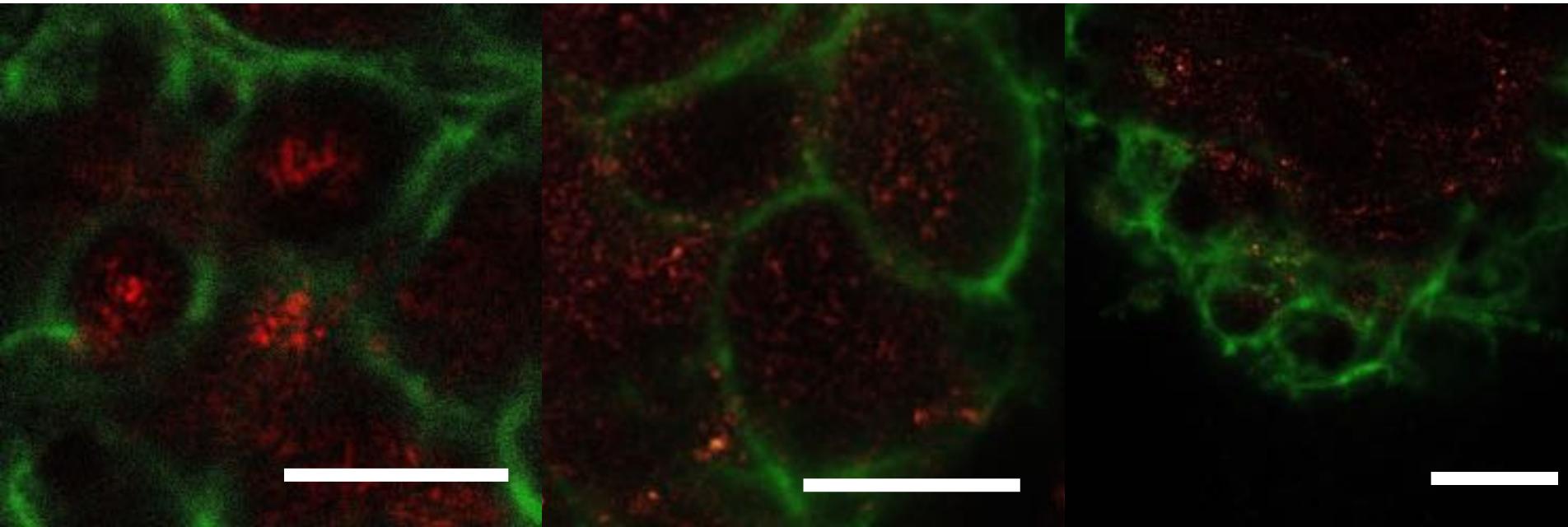
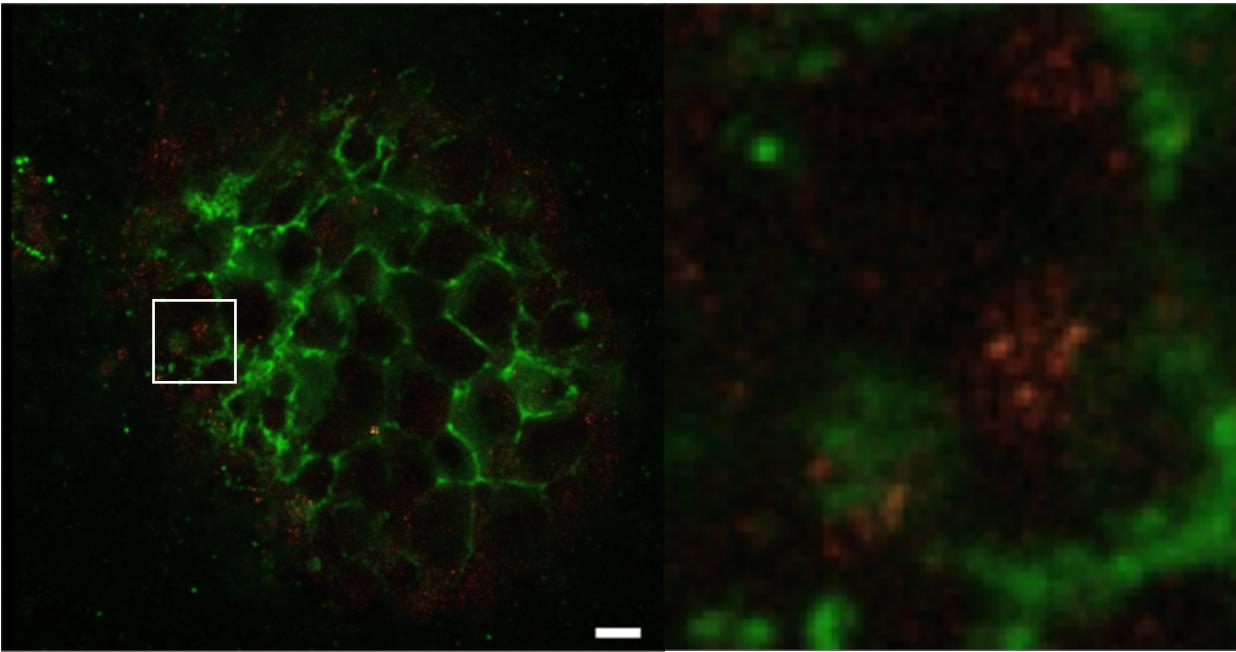
Particle uptake in patient-derived organoids



HAPEI MSNP RhoB
CD44 (secondary alexa 488)

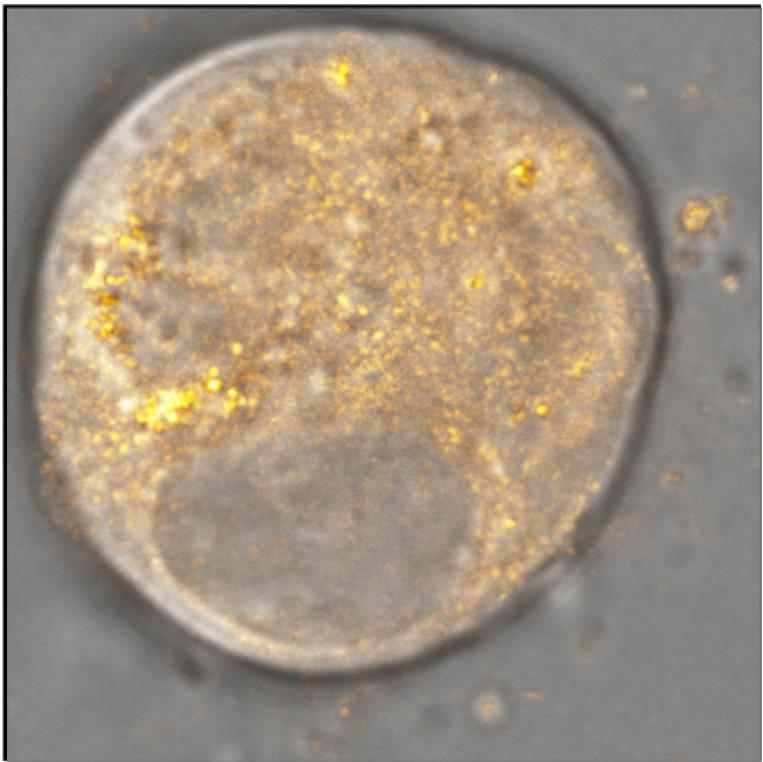
Scale = 10 um

Particle uptake in patient-derived organoids

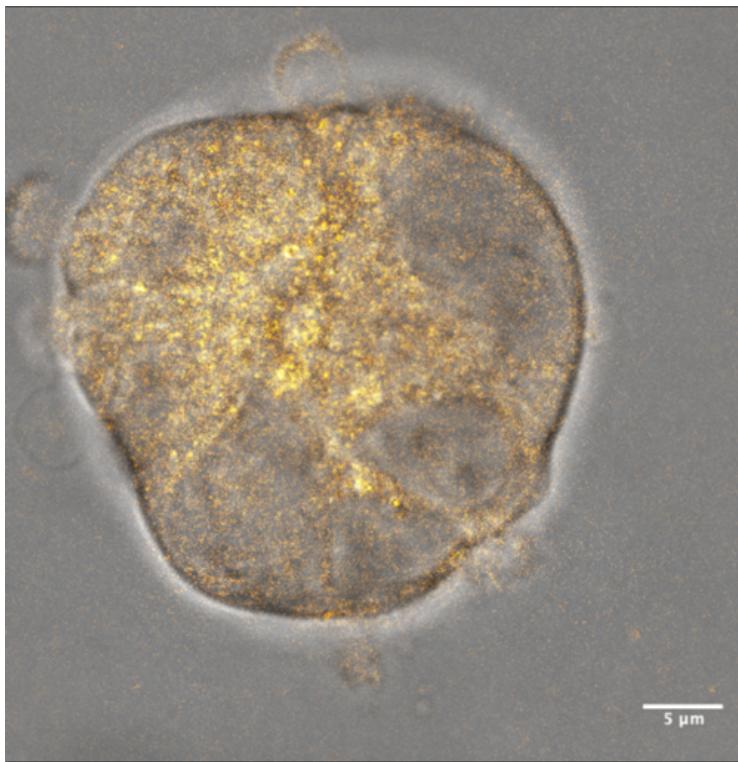


Scale = 10 um

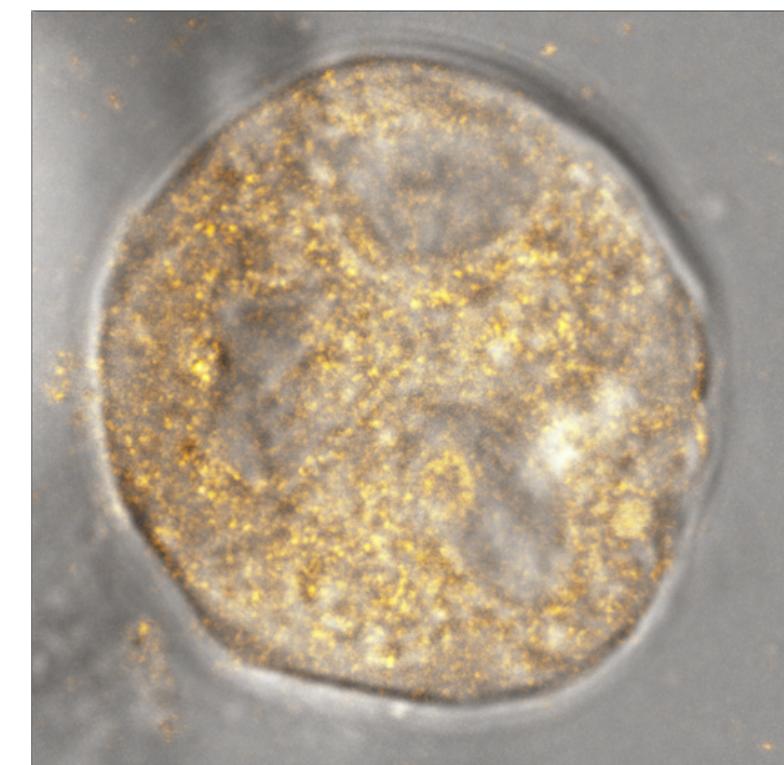
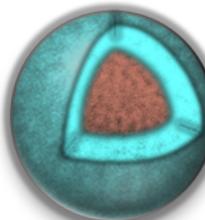
Particle uptake in patient-derived single cells



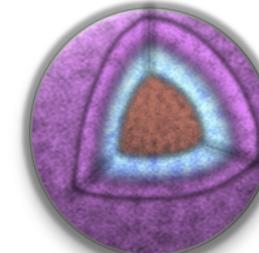
MSNPs



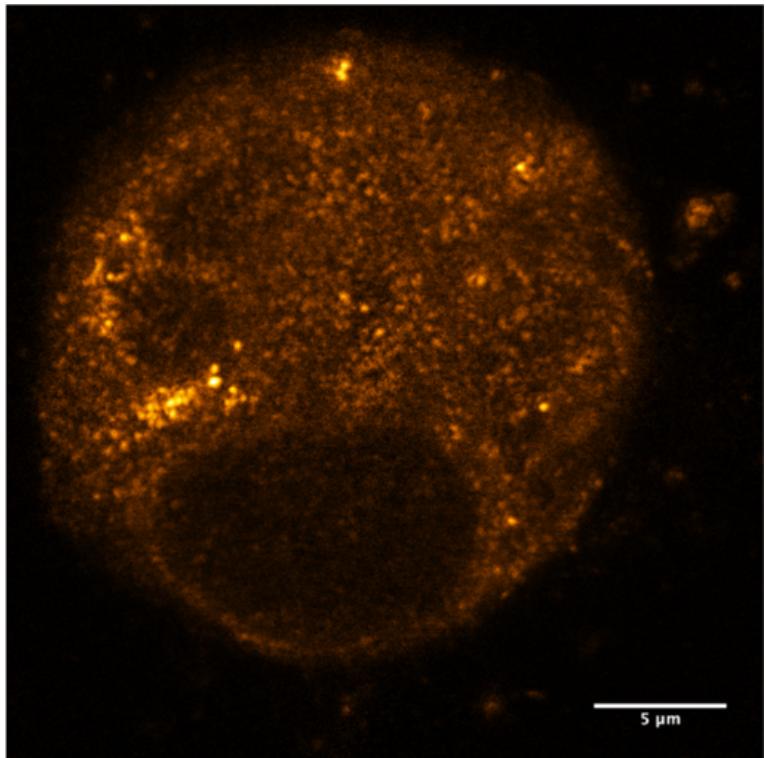
PEI-MSNPs



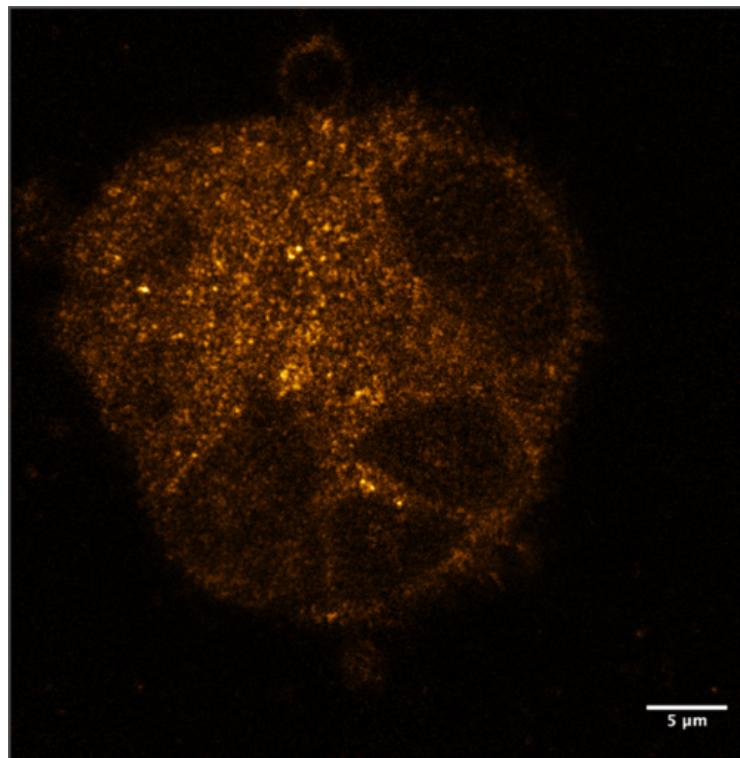
HAPEI-MSNPs



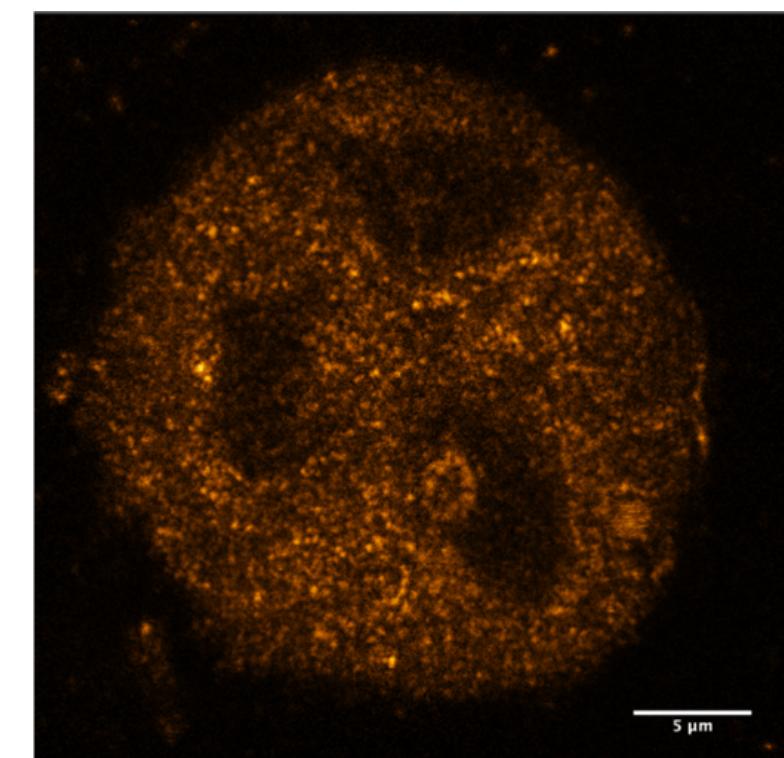
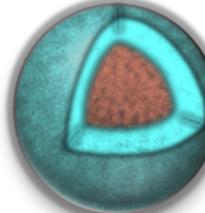
Particle uptake in patient-derived single cells



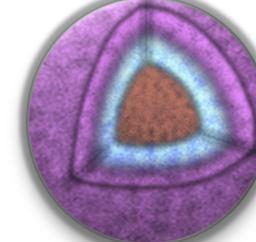
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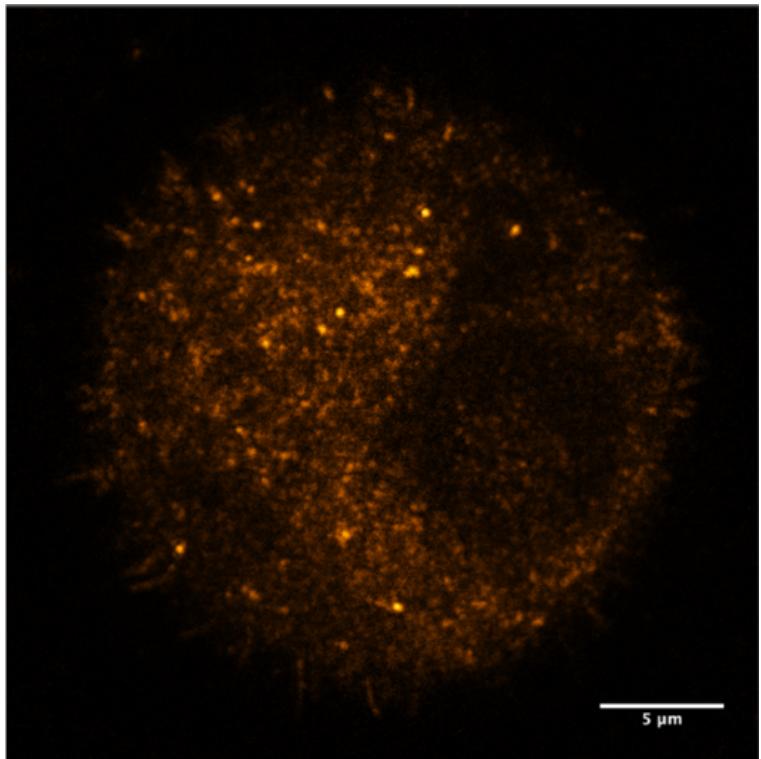
PEI-MSNPs



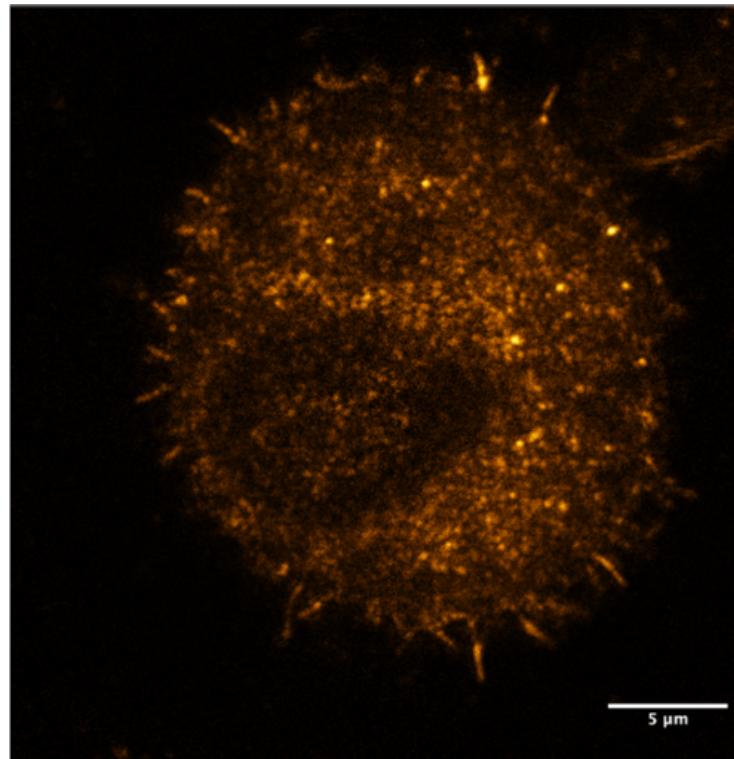
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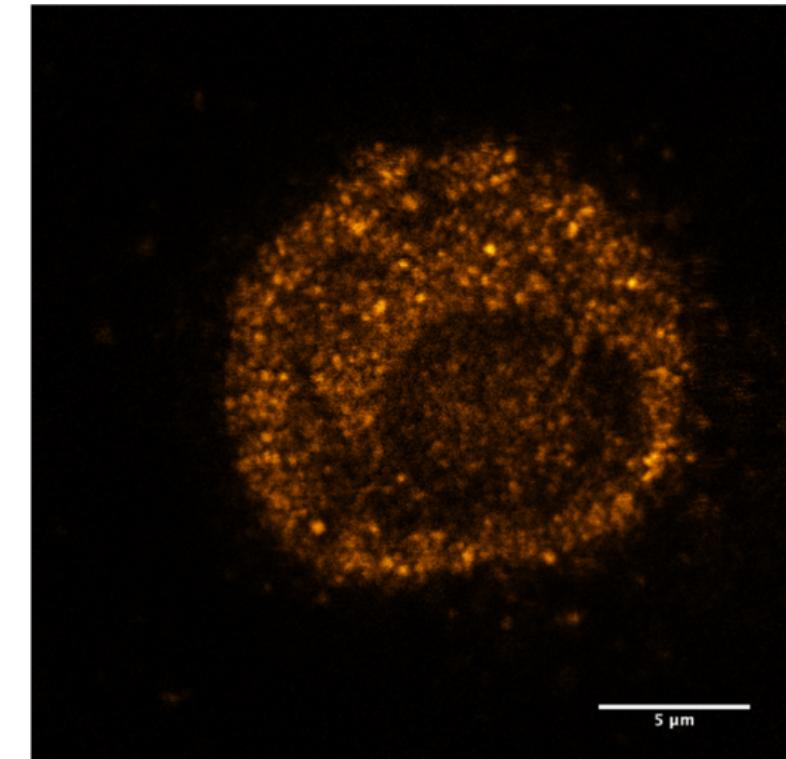
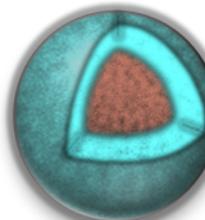
Particle uptake in A549



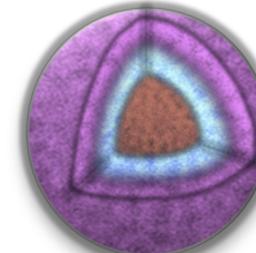
MSNPs



PEI-MSNPs

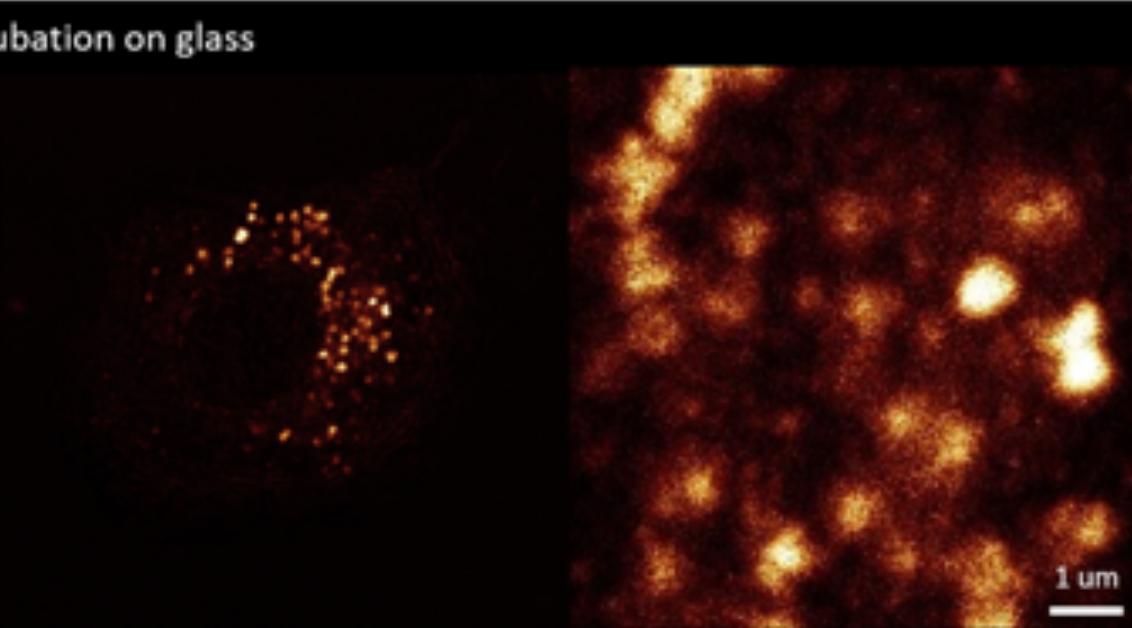
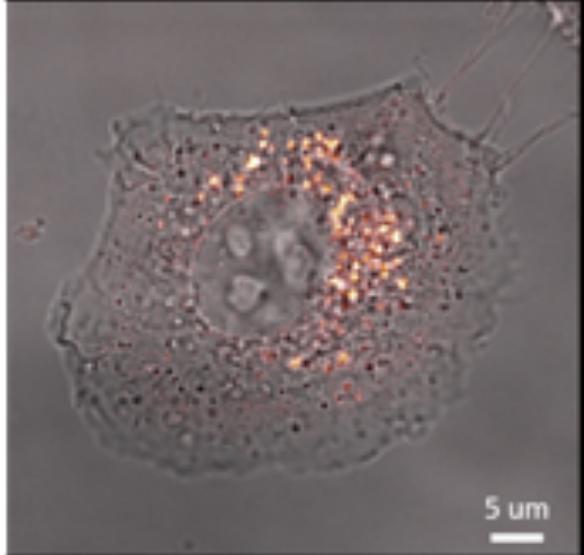


HAPEI-MSNPs

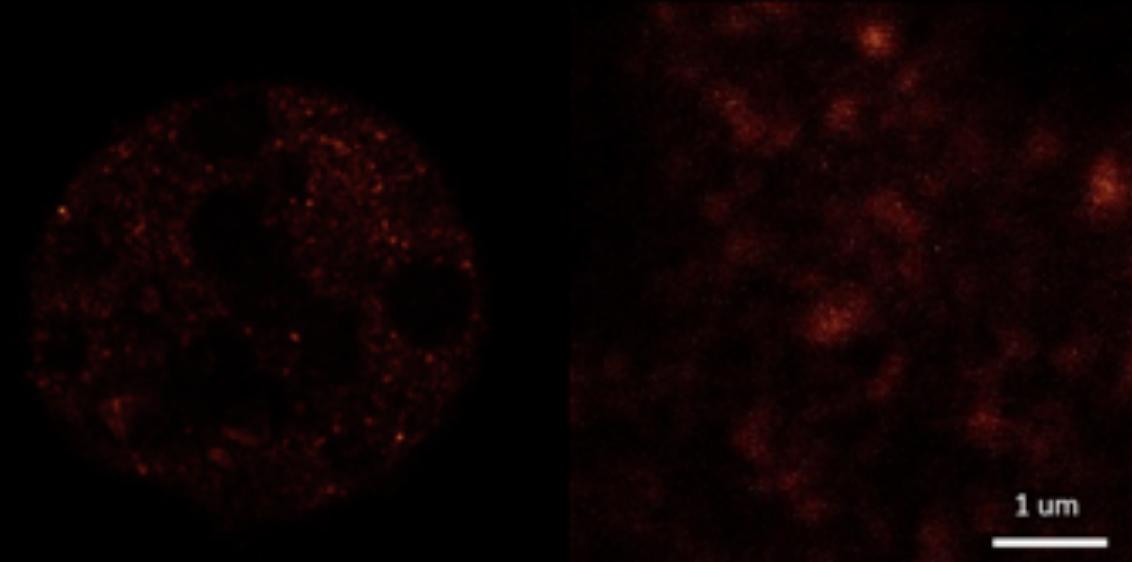
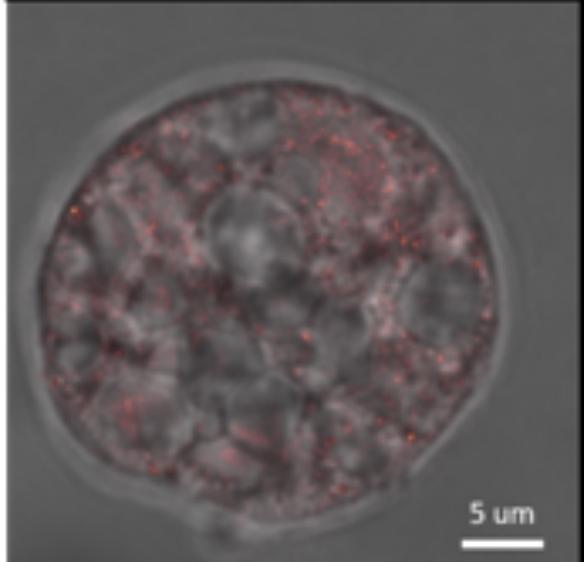


Particle uptake in A549

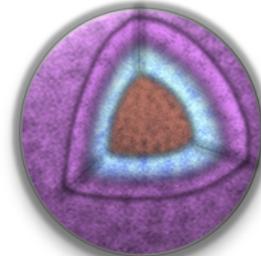
A549 24h HAPEIMSNP@RhoB incubation on glass

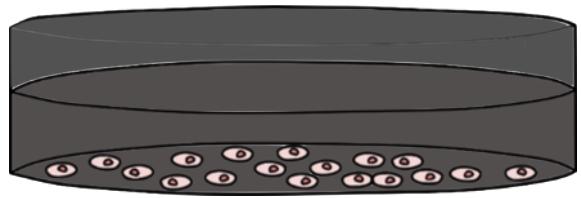


A549 24h HAPEIMSNP@RhoB incubation in Matrigel®



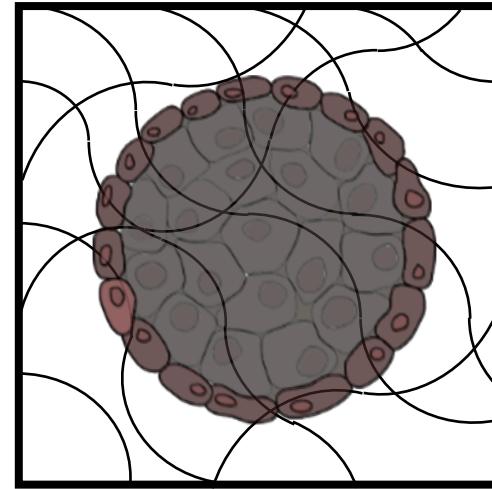
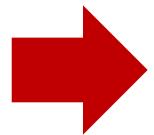
HAPEI-MSNPs





2D on glass (A549 cells)

Specific uptake of HA-coated NP



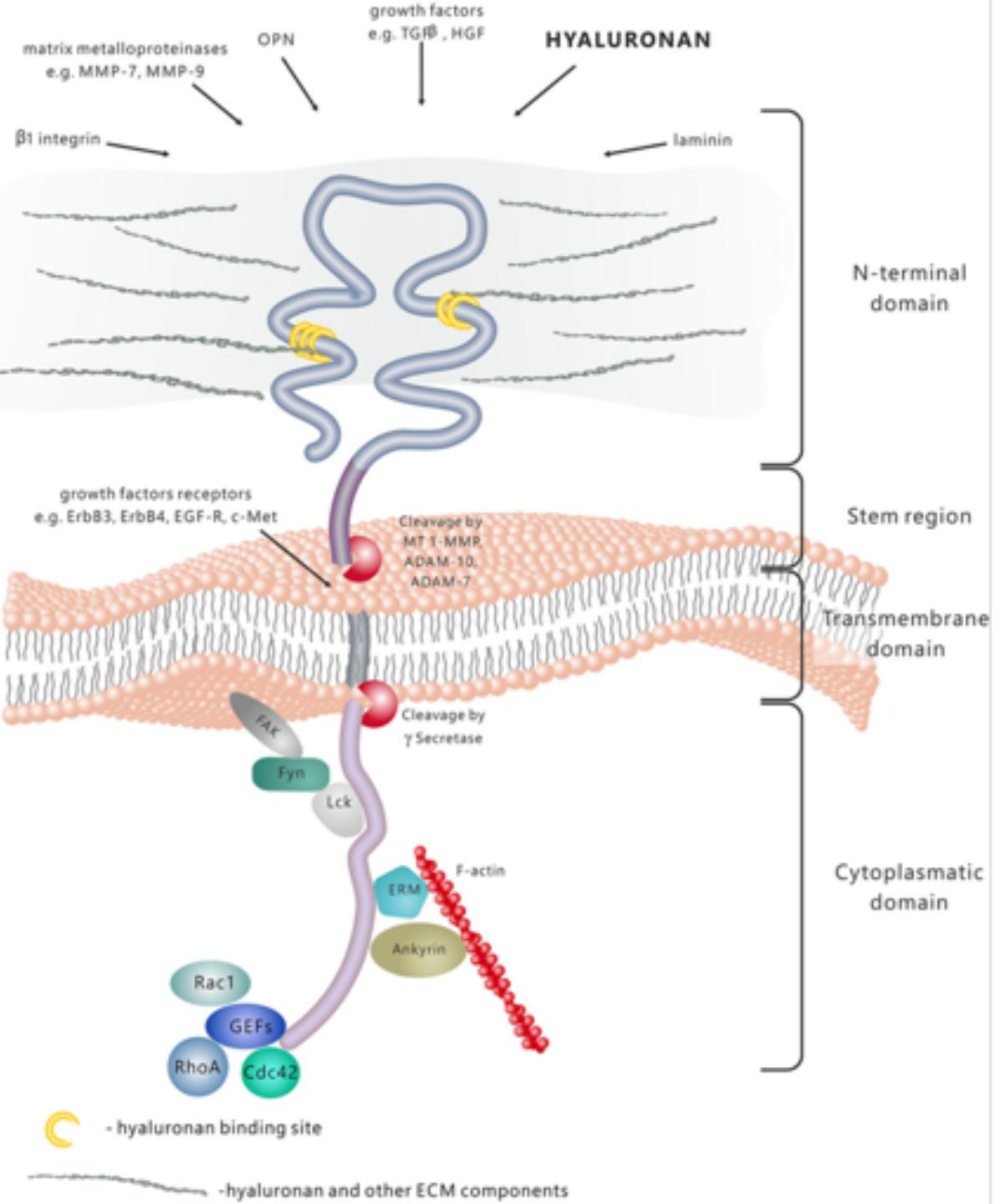
3D in Matrigel (organoids and A549)

Uptake of both HA-coated and non-coated particles

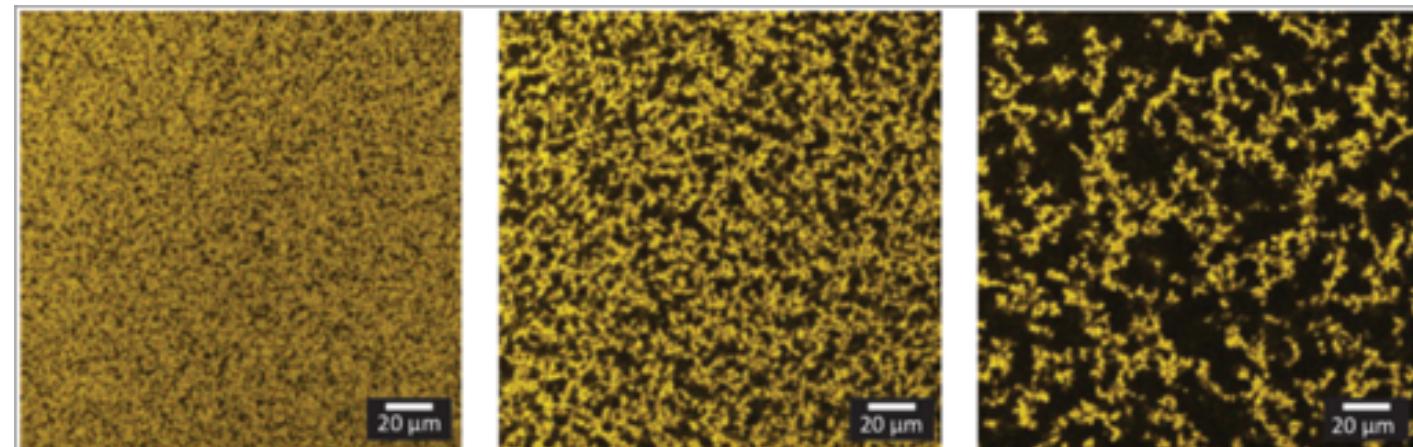
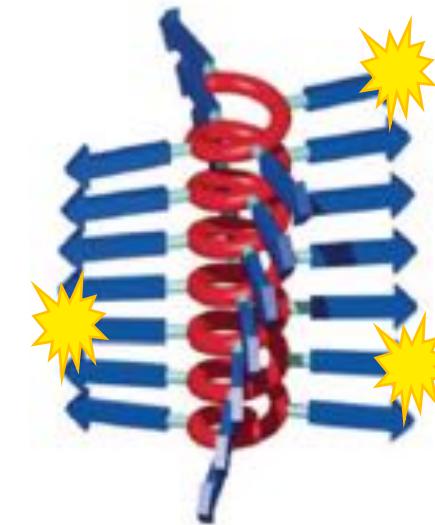
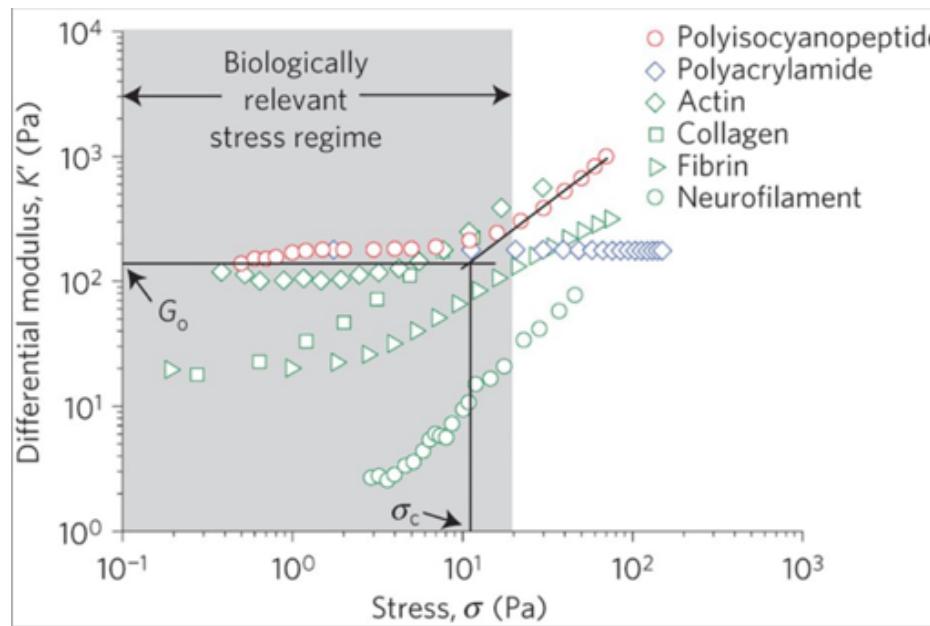
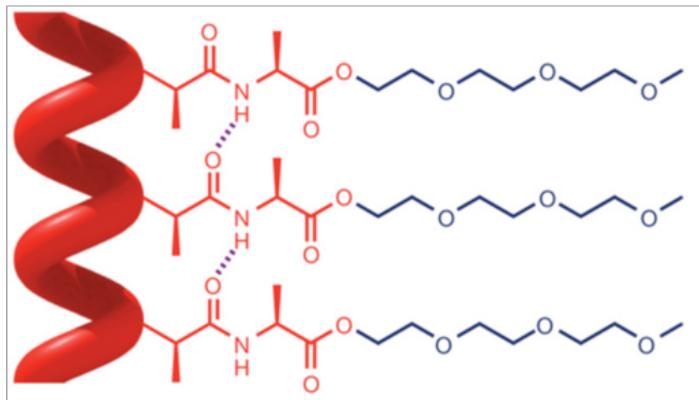


Role of CD44 receptor

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)



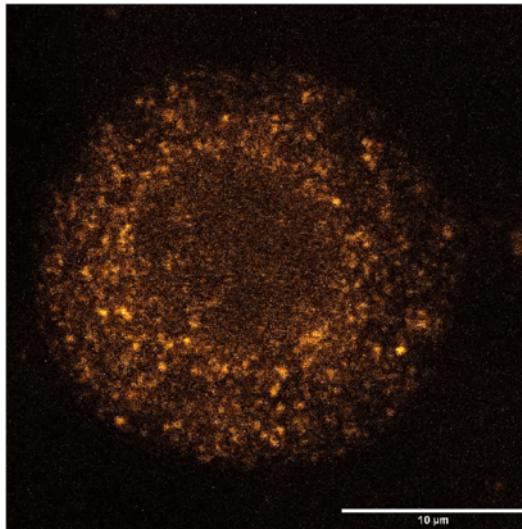
Nature, 493, 651-655 (2014), doi: 10.1038/nature11839

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

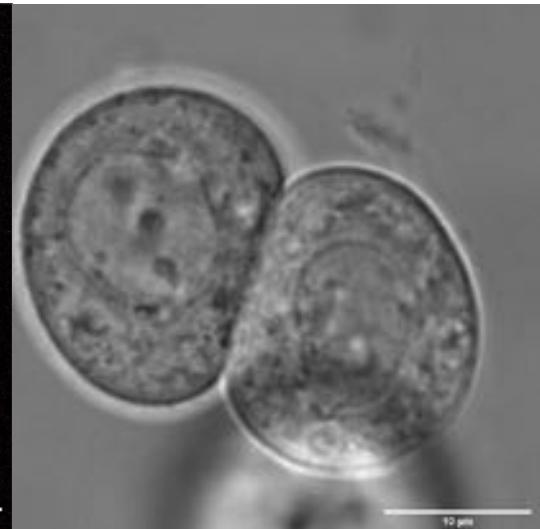
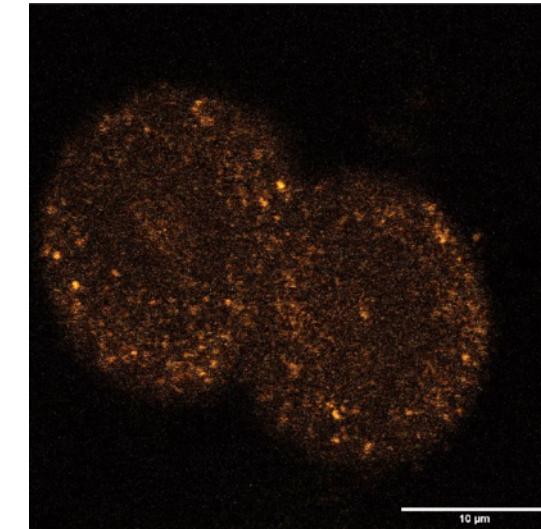
Particle uptake in 3D matrix

Matrigel

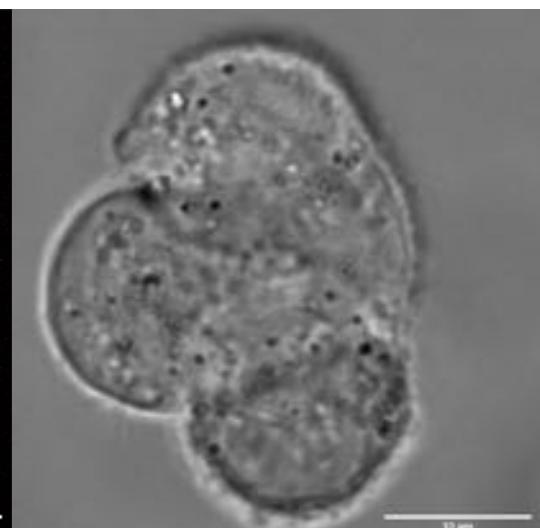
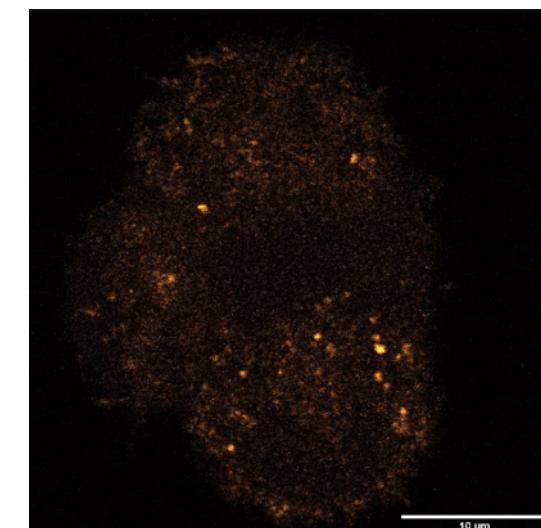
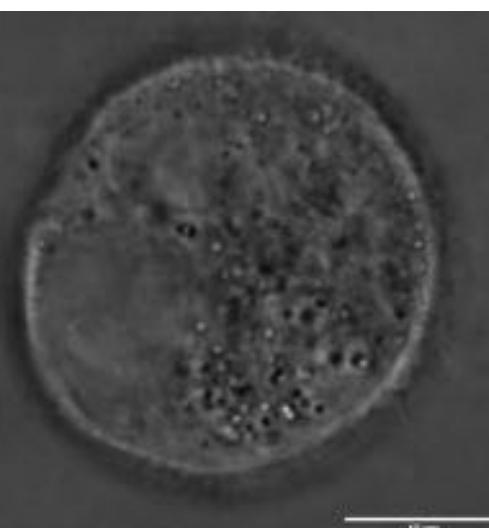
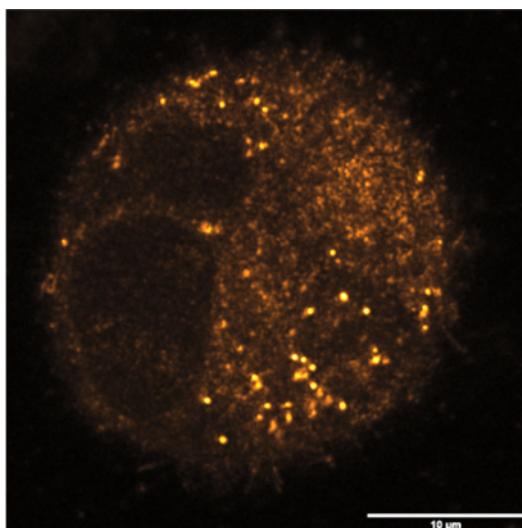
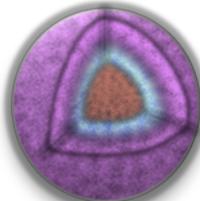
MSNPs



PIG-based hydrogel



HAPEI-MSNPs



Acknowledgments

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Drs. Monica Ricci

Prof. Dr. Johan Hofkens

Prof. Dr. Hiroshi Uji-i

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Prof. Dr. Paul Kouwer

Drs. Kaizheng Liu (Max, cells in PIC)

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Hasselt University

Prof. Dr. Jelle Hendrix

Instituto D. Carlos III (Madrid, SP)

Dr. Rodrigo Barderas

Drs. Guillermo Solis

? (Lisbon, PT)



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