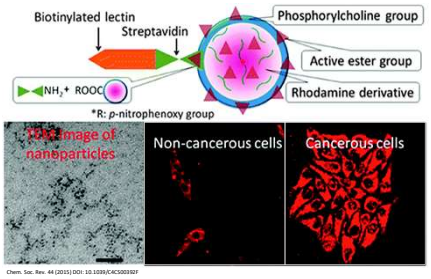
Workshop

Nanotoxicology in the context of the safety assessment of nanomaterials

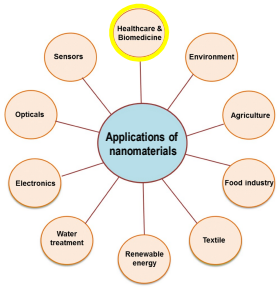
Using confocal microscopy for monitoring the subcellular impact of nanomaterials

Paulo Matos, PhD

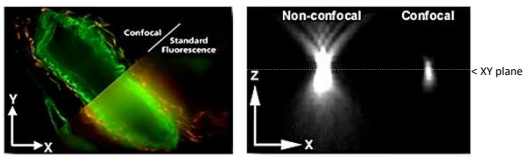
Fluorescence microscopy: a useful tool…



Nanomaterials: many applications…

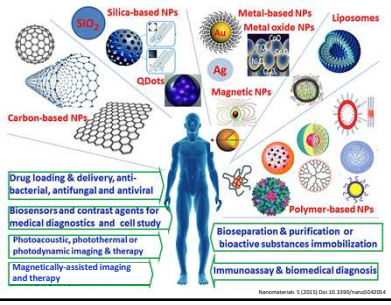


Comparison: Widefield - Confocal

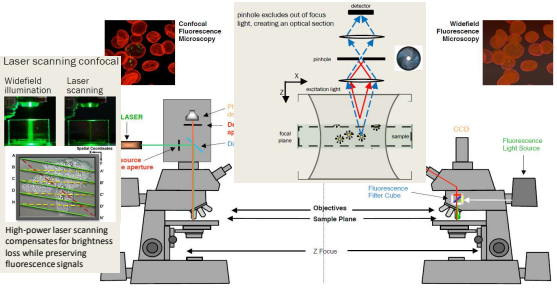


Higher z-resolution and reduced out-of-focus-blur make confocal pictures crisper and clearer.

Nanomaterials: biomedical applications…



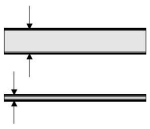
Comparison: Widefield - Confocal



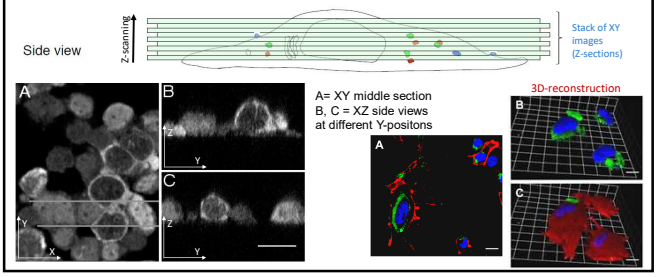
Comparison: Widefield - Confocal

***Increased** optical resolution in z*

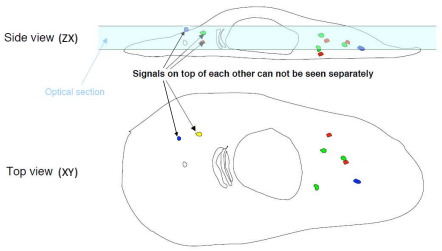
Widefield	2 - 3 μm
Confocal	< 0.5 μm



Confocal: Z-sectioning

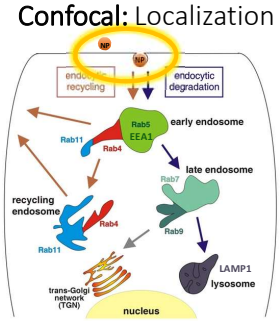


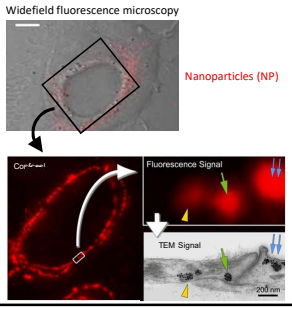
Widefield: optical section



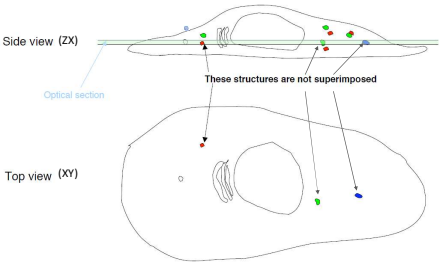
The precise location of a signal relatively to the 3D volume of the cell **cannot** be accurately determined!

Confocal: Localization of nanomaterials in cells



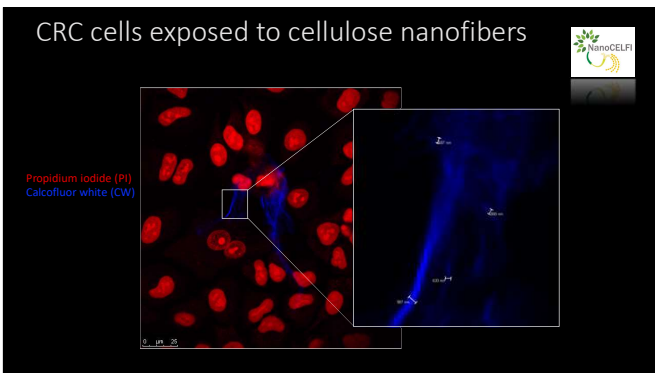


Confocal: optical section

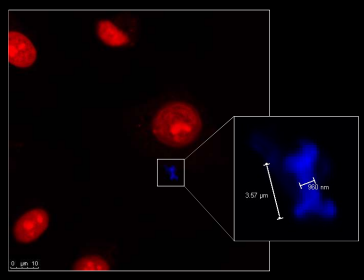


The location of a signal relatively to the 3D volume of the cell **can** be accurately determined!

CRC cells exposed to cellulose nanofibers

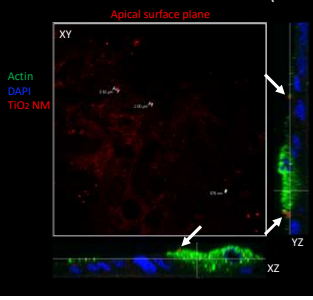


CRC cells exposed to cellulose nanofibers



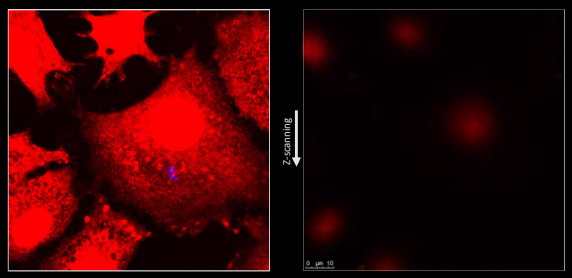
Propidium iodide (PI)
Calcofluor white (CW)

Polarized Caco2:HT29-Mtx co-culture exposed to titanium dioxide nanomaterials (TiO_2)



Actin
DAPI
TiO_2 NM

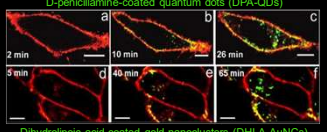
CRC cells exposed to cellulose nanofibers



Time course monitoring of live HeLa cells exposed to 2 different NMs

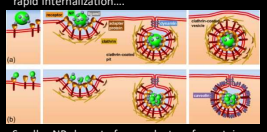
Cell membranes stained with CellMask DeepRed

D-penicillamine-coated quantum dots (DPA-QDs)



Dihydrolipoic acid-coated gold nanoclusters (DHLA-AuNCs)

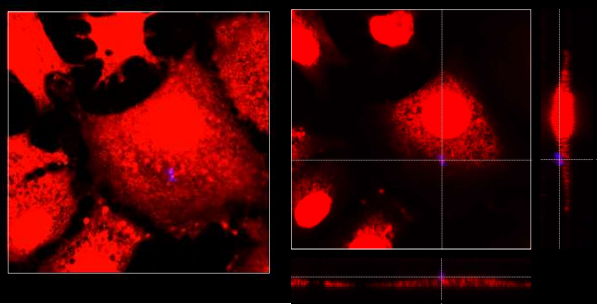
Larger NPs exert interactions with the cell membrane that are sufficiently strong to trigger rapid internalization....



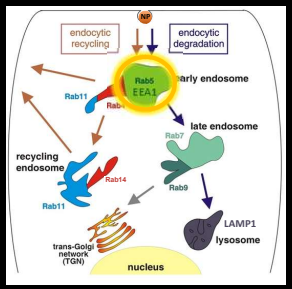
Smaller NPs have to form a cluster of a certain size to induce membrane invagination, delaying internalization...

Beilstein J. Nanotechnol. 5 (2014) DOI:10.3762/bjnano.5.248

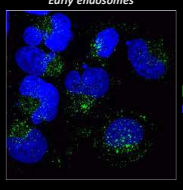
CRC cells exposed to cellulose nanofibers



What is the fate of internalized NPs?

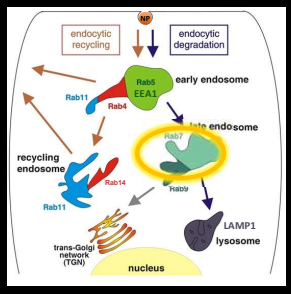


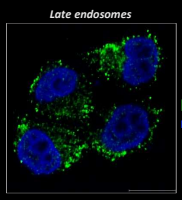
Early endosomes



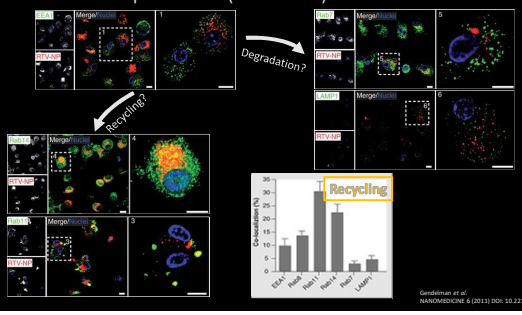
EEA1
DAPI

What is the fate of internalized NPs?

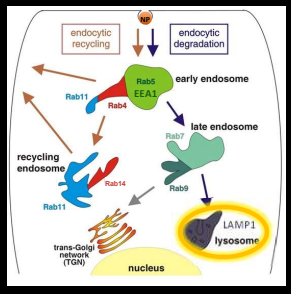


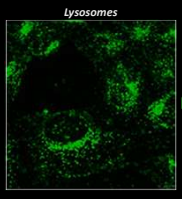


Following macrophages' endocytic trafficking of crystalline antiretroviral nanoparticles (RTV-NPs)…



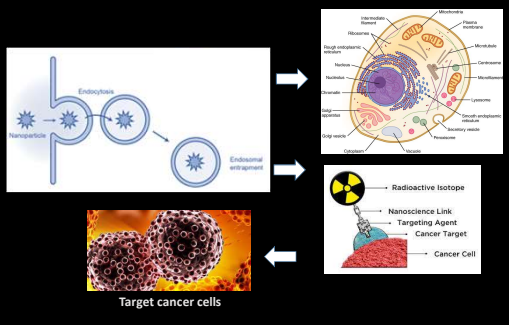
What is the fate of internalized NPs?



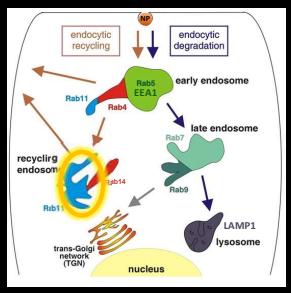


Most common route for internalized NMs, but…

Lysosomal entrapment of NMs…a road block?



What is the fate of internalized NPs?



How to monitor NMs' lysosomal escape?

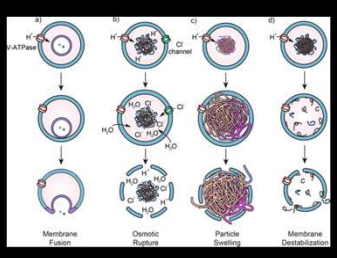
NMs can be developed or optimized to promote of endosomal escape:

(a) Membrane fusion between the nanoparticle structure and the endosomal membrane to release the cargo into the cytosol.

(b) The proton sponge mechanism where the NMs' buffering of lysosome pH increases the ionic influx of chloride osmotic lysing the lysosome

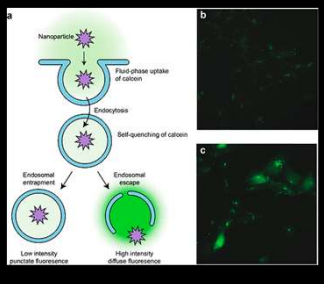
(c) Swelling of pH responsive nanogels ruptures the lysosomal membrane through mechanical strain.

(d) pH responsive nanoparticles disassemble and destabilize the lysosomal membrane.



BIOCONJUGATE CHEM 30 (2019) DOI: 10.1021/acs.bioconjchem.8b00732

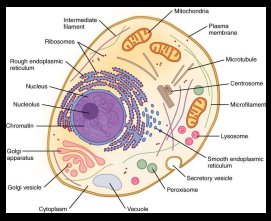
Confocal microscopy to monitor NMs' lysosomal escape…

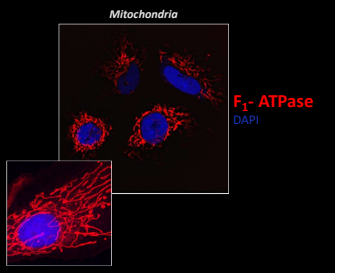


Dyes that change fluorescence intensity in response to a shift in pH (e.g., fluorescein, calcein): can be coupled to NPs to monitor their escape from acidic lysosomes to the cytosol (pH ~ 7.2) by fluorescence microscopy.

BIOCONJUGATE CHEM 30 (2019) DOI: 10.1021/acs.bioconjchem.8b00732

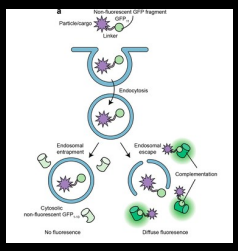
Co-localization with organelle-specific markers…

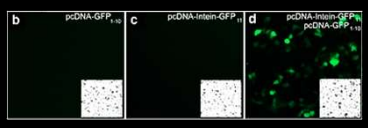




F_1- ATPase
DAPI

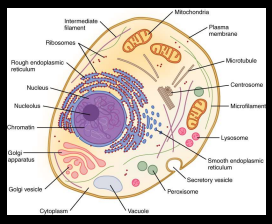
Confocal microscopy to monitor NMs' lysosomal escape…

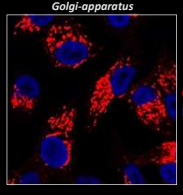




GFP complementation assay:
A small fragment of GFP is conjugated through a linker to a material. Fluorescence only appears if If the material escapes and complements with the large GFP fragment in the cytosol.

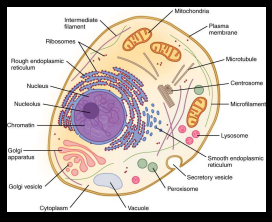
Co-localization with organelle-specific markers…

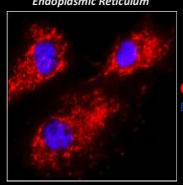




GM130
DAPI

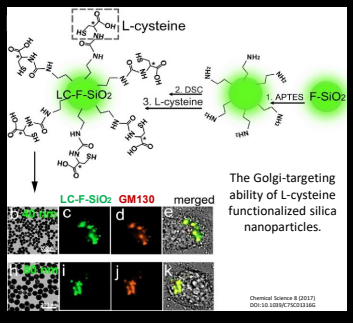
Co-localization with organelle-specific markers…





Calnexin
DAPI

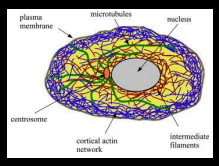
Co-localization with organelle-specific markers…

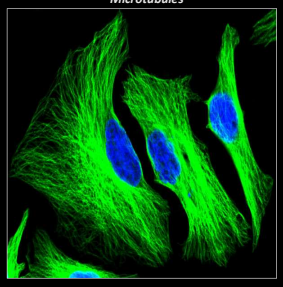


The Golgi-targeting ability of L-cysteine functionalized silica nanoparticles.

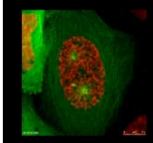
Chemical Science 8 (2017) DOI:10.1039/C7SC03316G

Visualizing NMs' interaction with the cytoskeleton…





Acknowledgements



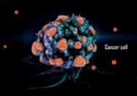
Toxicology Lab

Maria João Silva
Henriqueta Louro
Célia Ventura
Fátima Pinto
Dora Rolo
Ana Gramacho
Sara Teixeira

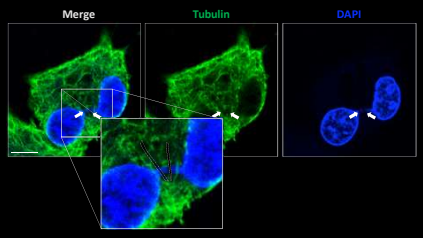
Oncobiology Lab

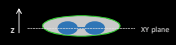
Peter Jordan
Joana Pereira

Funding



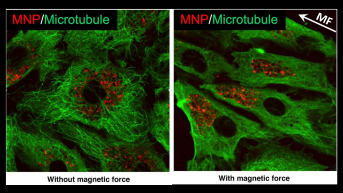
Carbon nanotubes cause DNA bridges in LC cells



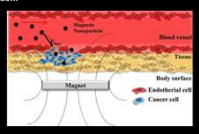


Manipulating microtubules to relax the endothelial barrier…

Fluorescent iron oxide nanoparticles bind microtubules in endothelial cells…



This makes the endothelial barrier "leaky" at specific sites, to allow drug molecules to pass into tissues…



Under a magnetic field (MF), the nanoparticles re-align the microtubule structure, temporarily disrupting cell–cell junctions…

Appl Nanosci 4 (2014) DOI: 10.1007/s13204-013-0216-y
https://www.genengnews.com/topics/drug-discovery/leaks-magnetically-induced-between-cells-could-promote-drug-delivery/

Using confocal microscopy for monitoring the subcellular impact of nanomaterials
Paulo Matos, INSARJ

The physicochemical properties of nanomaterials, such as their small size and high surface area ratio, make them ideal for many applications in industry and biomedicine. However, those same properties increase their ability to interact with cells and tissues, allowing their permeation through several biological barriers. While these abilities have been exploited in the development of novel drug-delivery systems, the widespread use of nanomaterials makes the evaluation of the potential cytotoxicity of their raw materials an important public health issue. In vivo studies are the usual gold standard when assessing compound toxicity, however, in vitro studies have also provided a lot of information regarding the toxicity and MoA of many compounds, and have proved crucial to clarify how the intrinsic and extrinsic properties of certain nanomaterials contribute to their interaction with cells an tissues. In this talk we will describe how confocal microscopy can be used in in vitro cell cultures to evaluate the subcellular impact of nanomaterials. We will point out the advantages and limitations of using confocal fluorescent microscopy in investigating how cells interact and react to the presence of different types of nanomaterial and how these can affect basic cellular functions.