Fluorescent labelling of nanoparticles for reliable bio-nano interactions study

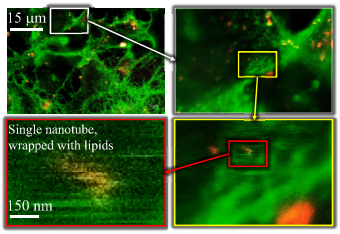
H. Majaron[1], B. Kokot[1], P. Umek[1], I. Urbančič[1,2], T. Koklič[1], M. Garvas[1] & J. Štrancar[1]

[1]Laboratory of Biophysics, Condensed Matter Physics Department, J. Stefan Institute, Ljubljana, Slovenia

[2]MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom, UK

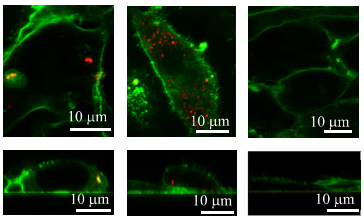
Motivation: understanding bio-nano interactions

Understanding the interactions between nanomaterials and the biological world is a crucial step in resolving nanotoxicological initiating events in adverse outcome pathways. By observing the initial nano-bio contact and localizing the nanomaterial inside the cell later on, plenty of insight into such interactions and their consequences may be gained. Hopefully, the knowledge on why and how certain nanomaterials have an adverse effect on biological systems, will one day lead to development and use of nanomaterial, non-toxic by design.



Membranes of living cells LA-4 (green) and TiO_2 nanotubes (red), seen with a STED microscope. Overlay is yellow.

The problem: labelling poses a challenge



sonication broke the nanotubes

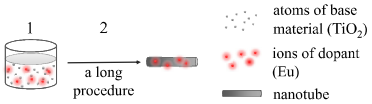
poorly washed free probe

probe desorbed from nanotubes

A common approach to nanomaterial localization in living systems is by means of fluorescence microscopy. To offer reliable results, fluorescent molecules (fluorophores) must be covalently bound to the cells and nanomaterial. Labelling cells is a breeze, whereas labelling nanomaterial poses a bigger challenge – its surface is usually not suitable for labelling with commercial fluorophores. Moreover, if not done carefully, poorly labelled nanomaterial will cause experimental artefacts.

Labelling approach 1: dope nanotubes with fluorescent atoms

The process

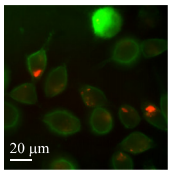


1. a solution of europium salt is added to the usual suspension for TiO_2 nanomaterial synthesis
2. a long multistep procedure (includes drying, washing, ion-exchange, and calcination) leads to formation of doped anatase TiO_2 nanotubes

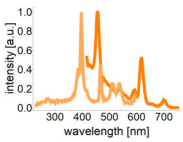
Characteristics of the material

+ no desorption of probe from nanomaterial
+ photostability
+/- no spectral sensitivity to local polarity, pH, …
+/- long lifetime
+/- narrow excitation and emission spectrum
- difficult and long synthesis
- large europium losses (5% → 0,1%)
? appropriate for high resolution imaging?

! doping must be performed during nanomaterial synthesis – not applicable to any nanomaterial



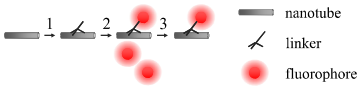
A confocal image of cells (green) incubated for a day with europium-doped TiO_2 nanotubes (red).



The narrow excitation (light orange) and emission (orange) spectrum of europium-doped anatase TiO_2.

Labelling approach 2: label nanotubes with fluorescent molecules

The process[1]

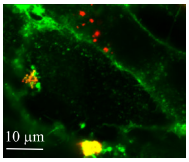


1. a silane linker (AEAPMS) is bonded to dispersed TiO_2 nanotubes
2. fluorescent probe (Alexa 647 NHS) is covalently bonded to the linker
3. the excess free probe is filtered away

Characteristics of the material

+ any nanomaterial can be labelled with any probe
+/- spectral sensitivity to local polarity, pH, …

! possible linker desorption from nanomaterial
! organic probes are enzyme-digestible
! choose the right probe for the job – it should have an appropriate charge, polarity and membrane interaction factor (MIF)[2]



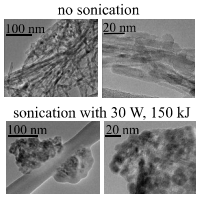
A STED image of cells (green) incubated for a day with labelled TiO_2 nanotubes (red).

Take-home message:

label your nanoparticles wisely and with care

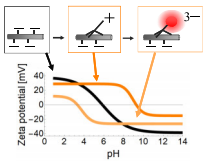
Labelling quality control: What could possibly go wrong?

Changes in nanoparticle morphology



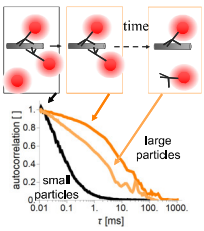
Excessive sonication and/or too concentrated dopant change the morphology and/or crystal lattice structure of the nanoparticle (TEM).

Changes in surface charge of nanoparticles



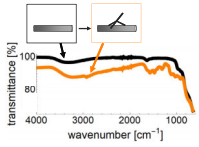
Binding of linkers and fluorescent probe to the nanoparticle surface change its surface charge (Zeta potential).

Unwashed and/or desorbed probe



Poorly washed unbound probe and uncharacterized desorption of probe from nanomaterial increase background and induce artefacts[3] (FCS, fluorometric measurements).

Unsuccessful functionalization



Unsuccessfully functionalized nanomaterial is a recipe for disastrous labelling (FTIR).

Hungry for more?

[1] Garvas, M. *et al.* Protein Corona Prevents TiO2 Phototoxicity. *PLOS ONE* **10,** e0129577 (2015).

[2] Hughes, L., Rj, R. & Sg, B. Choose your label wisely: water-soluble fluorophores often interact with lipid bilayers. *PLOS ONE* **9,** e87649 (2014).

[3] Tenuta, T. *et al.* Elution of Labile Fluorescent Dye from Nanoparticles during Biological Use. *PLOS ONE* **6,** e25556 (2011).

Thank you,

Zoran Arsov, Rok Podlipec and Žiga Urh for smart questions and comments, Katarina van Midden and David Dolhar for a helping hand in the lab, and Tobias Stöger and Raphaël Prungnaud for helping us get started with alveolar cells

Laboratory of Biophysics Condensed Matter Physics Jožef Stefan Institute

SmartNanoTox Smart Tools for Gauging Nano Hazards

European Commission | Horizon 2020 European Union funding for Research & Innovation