Assessment of multiple dye doped core-shell silica nanoparticles uptake in U937 cell line

Federica Sola[1,2], Barbara Canonico[1], Erica Cesarini[1], Maria Gemma Nasoni[1], Chiara Pellegrino[2], Chiara Barattini[2], Alfredo Ventola[2] and Stefano Papa[1]

[1]*Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino (PU), Italy*

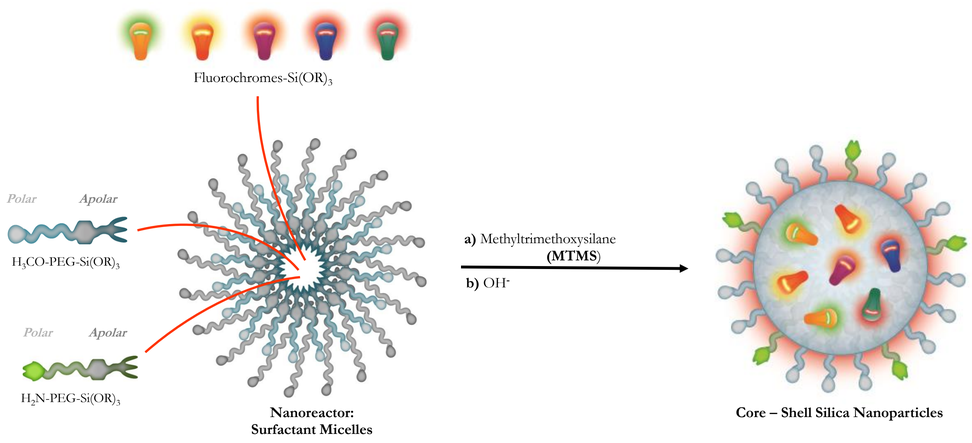
[2]*AcZon s.r.l., Monte San Pietro (BO), Italy*

INTRODUCTION

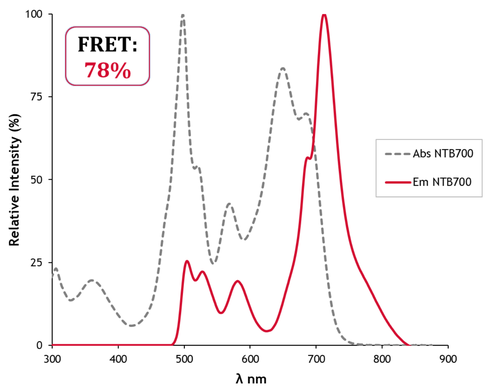
Despite the widespread use of traditional fluorochromes, their limits are well known; therefore, the identification of new technologies, allowing to overcome those limits, is nowadays a main diagnostic challenge. Thanks to their unique characteristics, fluorescent nanoparticles (NPs) have been extensively studied as candidates in several biomedical applications. AcZon NPs are dye doped core-shell silica nanoparticles that allow to overcome biological molecules limits. Concerning fluorescence, silica has proven to be an excellent tool: it's photophysically inert, it's not involved in energy or electron transfer processes and, moreover, it's intrinsically non-toxic. The most innovative feature of these fluorescent NPs is the ability to be a platform where the energy transfer process, known as FRET, occurs at a high efficiency rate, increasing the Stokes shift. Among all the different kinds of available fluorophores, a particular interest is addressed to red and near-infrared emitting dyes, especially for imaging applications because of their reduced interaction with tissues, decreasing damages to living cells. Furthermore, these wavelengths have an enhanced tissue penetration and, as a consequence, a reduced cells autofluorescence, allowing a better images resolution due to a lowered background.

The aim of the present study is the NPs uptake investigation in a cellular model, U937 (monocyte-like human histiocytic lymphoma cell line), for their future *in vivo* applications.

NPs SYNTHESIS & FRET



AcZon NPs are core-shell dye doped silica nanoparticles synthesized through a micelle-assisted method, where a surfactant is used to create a nanoreactor within which reagents arrange. The base-catalysed hydrolysis of different trialkoxysilanes leads to the formation of monodisperse NPs.



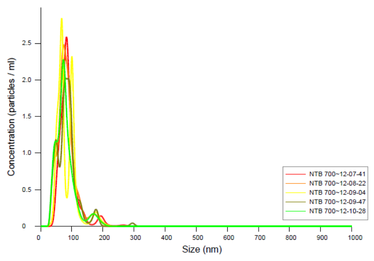
FRET efficiency has been calculated considering the emission intensity of the final acceptor and average intensity of donors.

Shell is composed by two different polyethylene glycols (PEG), both terminating with a trialkoxysilane group:

- H_3CO-PEG-Si$(OR)_3$: main component of the shell. Induces stability and solubility in water;
- H_2N-PEG-Si$(OR)_3$: allows the presence of amine reactive groups on the external shell.

To obtain nanoparticles excitable with blue laser and that emit in the near-IR (**NT_B700**), five different dyes were selected to be simultaneously entrapped into silica core. The strong interconnection reached inside the core leads to a good FRET.

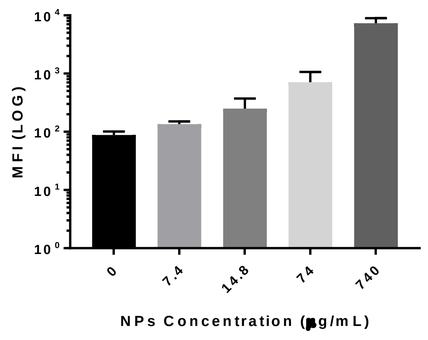
A preliminary NPs size characterization (diameter of 84.1 +/- 1.5 nm) was performed using Nanosight LM10 Nanoparticle Tracking Analysis (Malvern Panalytical).



NPs UPTAKE MEASUREMENT

U937 cells ($5*10^5$ cells/well) were seeded into 6-well plate and incubated from 1 to 72 hours to evaluate NPs cytotoxicity using trypan blue exclusion assay. There was no significant difference in cell viability between control and cells treated with NT_B700 NPs. Data confirmed by flow cytometry analysis with Propidium Iodide (PI) staining.

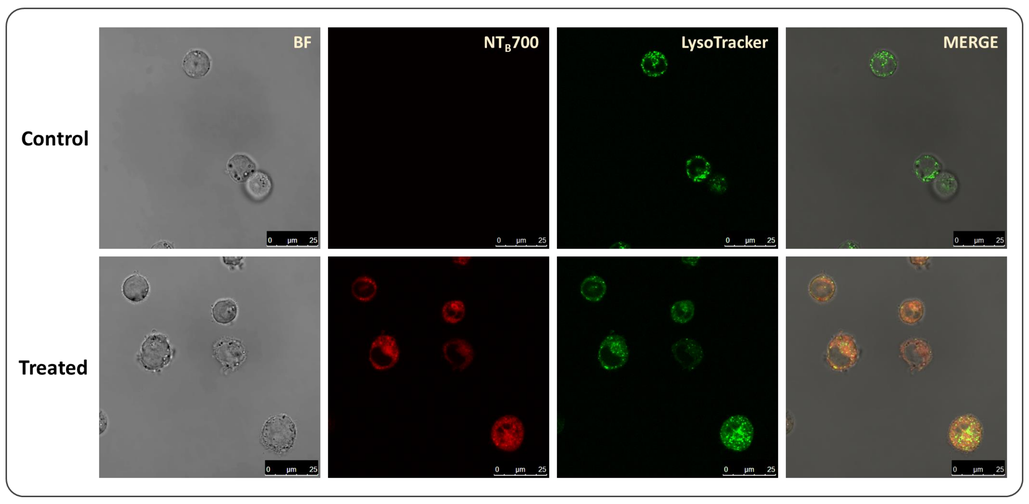
To characterize how NPs are taken up by cells, we quantified their incorporation through flow cytometry, measured by mean fluorescence intensity (MFI). Cytometric experiments were carried out with a FACSCanto™ II flow cytometer (BD Biosciences).



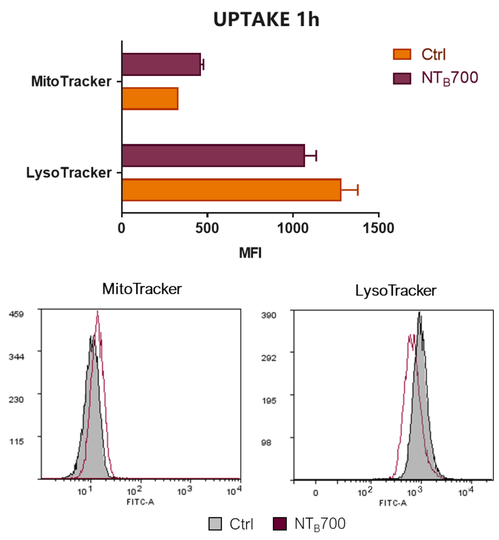
NT_B700 NPs were already internalized after 30 minutes of incubation at 37 °C and no differences in terms of MFI under longer incubation times (60', 120', 180') were observed. It has been known that NPs concentration can impact uptake efficiency, therefore we evaluated concentration dependence in NT_B700 NPs uptake. U937 cells were incubated for 1 hour at 37 °C with different concentrations of NPs (7.4, 14.8, 74 and 740 µg/mL). The histogram clearly shows a concentration-dependence, but we didn't investigate if there could be a saturation of the system.

LYSOSOMES & MITOCHONDRIA INVOLVEMENT

In order to investigate whether NT_B700 NPs altered host lysosomes integrity, after 1 hour of incubation at 37°C with NPs, U937 were stained with LysoTracker® Green (LTG) and detected by flow cytometry and confocal microscopy. Fluorescence microscopy analysis were performed by a Leica TCS SP5 II confocal microscope (Leica Microsystem). NT_B700 NPs induced a concentration-dependent reduction of LTG MFI, the lowest concentration of NPs (7.4 µg/mL) showed similar values to untreated cells. Confocal analysis confirmed what have been observed by flow cytometry. The underlying images show a lower and less "spotted" fluorescence in U937 cells which internalized NPs, compared to control cells, showing the involvement of lysosomal pathway after NT_B700 spontaneous uptake.



After 1 hour NT_B700 NPs exposure, cells were additionally stained with MitoTracker® Green (MTG) to evaluate a possible alteration of mitochondria state. Confocal analysis showed that mitochondrial shape appeared to be somewhat changed compared to control cells. The side graph shows a slight variation of MTG fluorescence intensity in U937 cells which internalized NPs. These data have been collected after 1 hour of treatment; therefore, it will be necessary extend these analysis to at least at 24 hours of incubation which is a physiologically relevant time point.



CONCLUSIONS

Results showed not significant cytotoxicity induced by the aforementioned NPs. The NT_B700 NPs uptake in U937 cell line is a concentration-dependent spontaneous rapid process that involves lysosomes and modifies mitochondria morphology. Despite the observed involvement of these organelles, the evaluation of which endocytic mechanism is exploited for NPs internalization and a more detailed characterization of their intracellular fate might be required for future applications. NPs characterization is a main goal in order to employ this new technology in biomedical purposes, in particular in diagnostic field or as drug carriers.

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If you are interested in more information, don't hesitate to contact us.

Tel. +39 051/6759711

Fax +39 051/6759799

www.aczonpharma.com

federicasola@aczonpharma.com

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