Intracytoplasmic staining procedure using new fluorescence stable probes based on Nanoparticles technology

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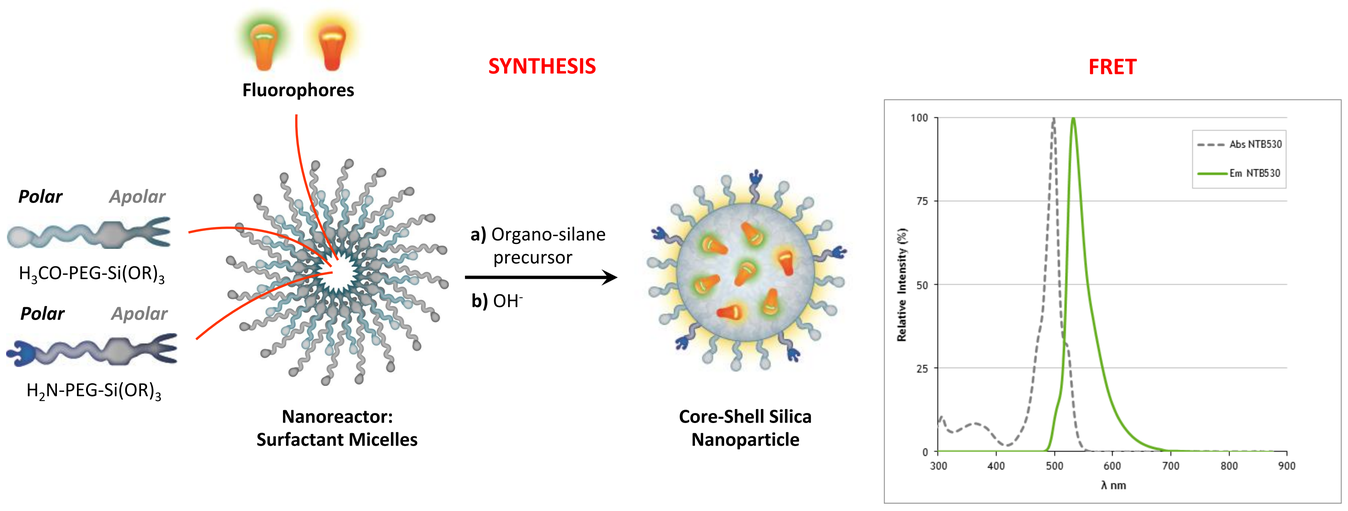
Introduction

Conjugation of biological molecules with fluorescent dyes has taken growing interest thanks to the huge number of exploitable biological applications. In order to increase sensitivity and selectivity, dyes are required to show high brightness and photostability. While classical dyes are still affected by lack in stability and low intensity emission in water solution, fluorescent nanoparticles (NPs) are emerging as promising probes for biological applications. In particular fluorescent silica nanoparticles help to overcome these limitations by protecting the fluorophore within their own matrix. Silica also offers many advantages being transparent to UV light, biocompatible, non toxic, inexpensive and extremely versatile.
In this study our purpose is to investigate the behaviour of NPs conjugated with Ms. anti human Pan-cytokeratin in intracytoplasmic staining and to evaluate their photostability.

Synthesis and FRET phenomenon

Aczon NPs are core-shell dye doped silica nanoparticles synthesized through a micelle-assisted method. All reagents are mixed together in a solution of water and n-butanol. Surfactant creates micelles within which all reagents, due their hydrophobicity, arrange. Dyes are themselves component of the silica core, thanks to a covalent modification with a trialkoxysilane group. Dyes with the opportune photophysical characteristics can generate an efficient fluorescence resonance energy transfer (FRET). The NPs used in these experiments are called NT_B530: they contain two different bodipy dyes which can be excited by common blue laser.

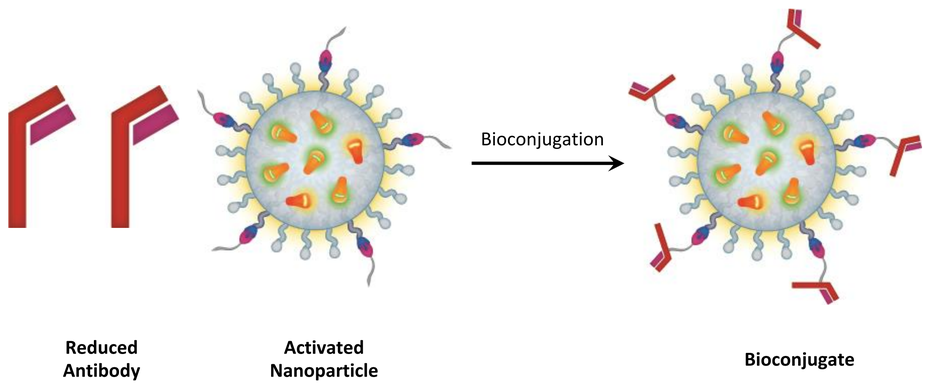


Site specific conjugation of antibody with NPs

Malemide-activated NPs were conjugated with an antibody to create a highly fluorescent system. This system preserves the specificity capabilities of molecular recognition antigen-antibody. The antibody used was Ms. anti human Pan-cytokeratin (PanCK), clone C11.

BIO-CONJUGATION PHASES

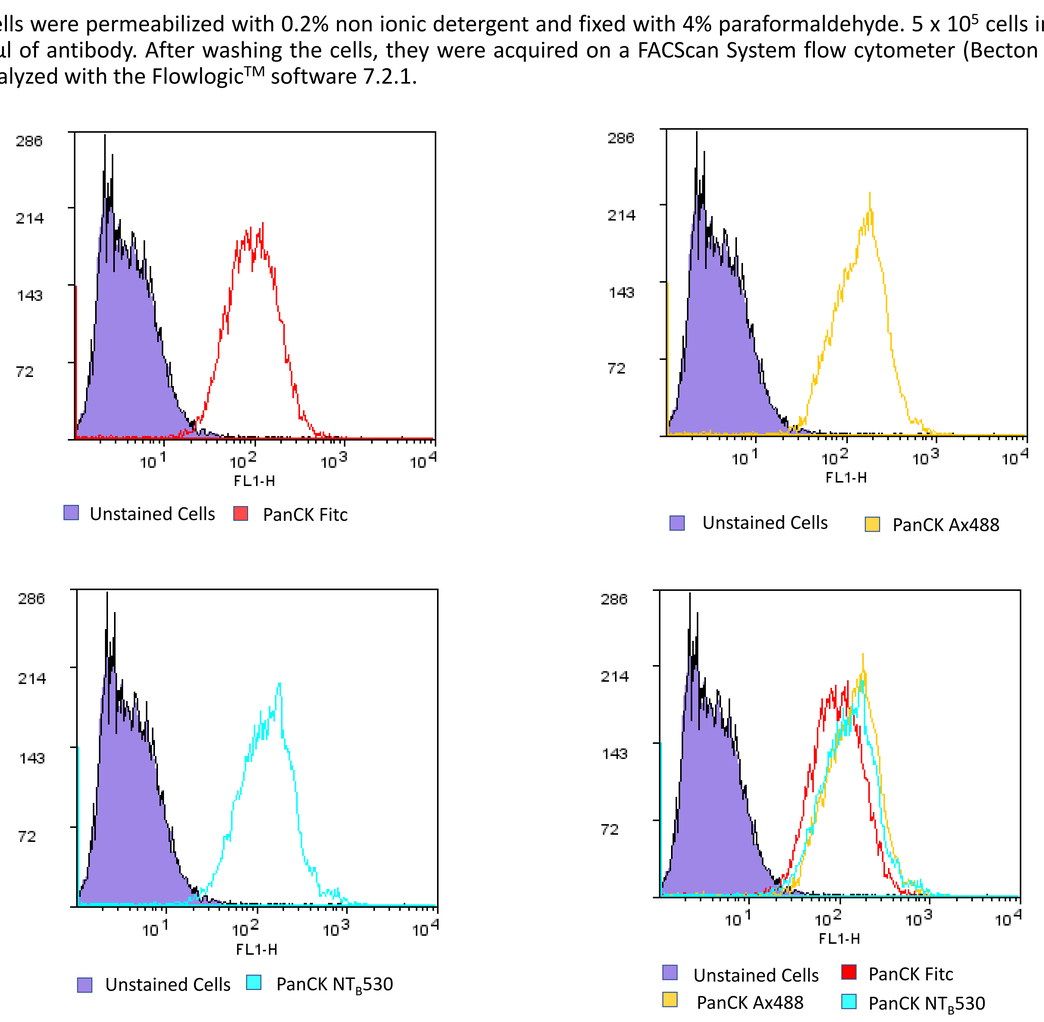
- NPs activation with a crosslinker
- Antibody reduction
- Conjugation of the two species
- Chromatographic purification



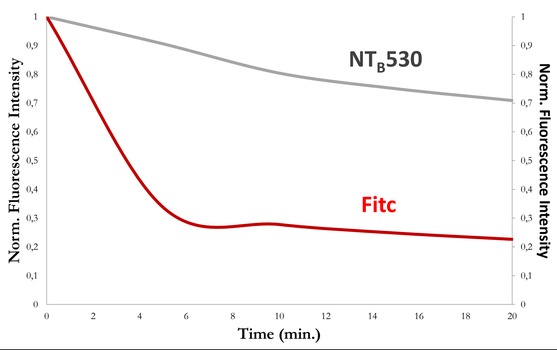
Evidence and results

Intracytoplasmic staining and flow cytometry evidence

MCF7 cells were permeabilized with 0.2% non ionic detergent and fixed with 4% paraformaldehyde. 5 x 10^5 cells in 100µl were stained with 10µl of antibody. After washing the cells, they were acquired on a FACScan System flow cytometer (Becton Dickinson). The data were analyzed with the Flowlogic™ software 7.2.1.

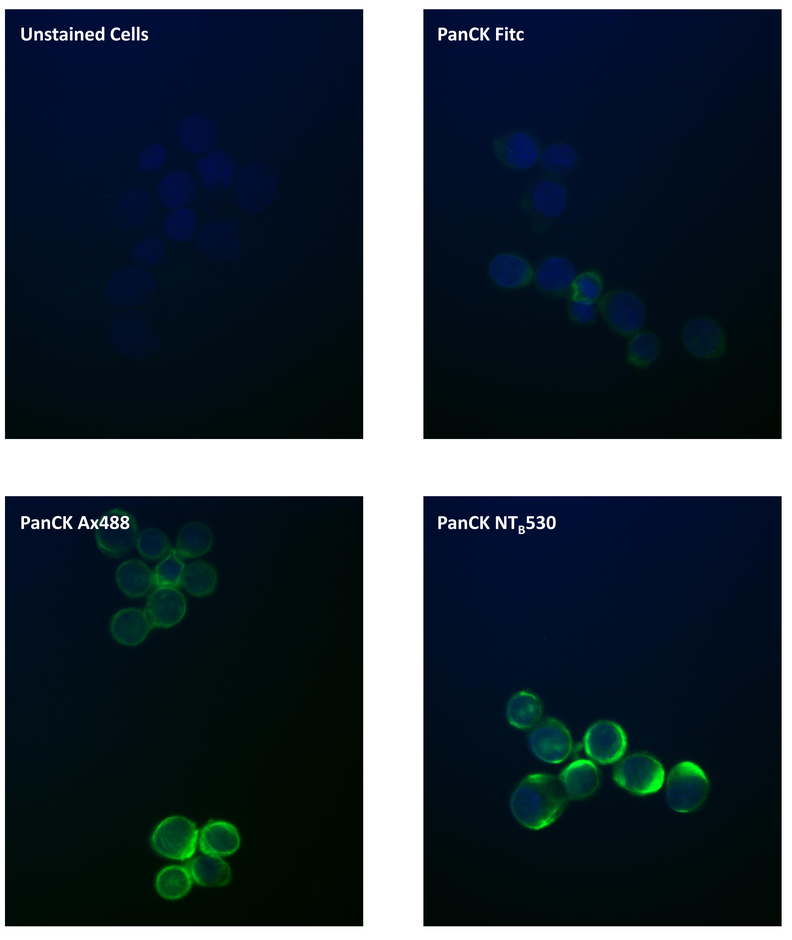


Comparing the Mean Fluorescence Intensity (MFI) of the antibody conjugated with NPs to Fitc or AlexaFluor™488 (Ax488), flow cytometry analysis showed a good NPs fluorescence (MFI: 151), better than Fitc (MFI: 115) similar to AlexaFluor™488 (MFI: 160).



Fluorescence microscopy evidence

MCF7 cells have been observed through fluorescence microscopy immediately before flow cytometric acquisition. The nuclei have been counterstained with Hoechst 33342.



The microscopy check confirmed the data collected in flow cytometry.

Photostability

The increased stability of NPs has been shown under stressing condition. The NPs were embedded in a thin polyacrylamide (10%) gel matrix. The doped gel was continuously irradiated under a fluorescence microscope, collecting images every 5 minutes. The photostability experiments underline a decrease of fluorescence intensity for the molecular fluorophore FITC (≈70%) in the first 20 minutes which isn't observed in the nanoparticles based fluorophore (loss of fluorescence intensity less than 30%).

Conclusions

The Pan-cytokeratin-NT_B530 conjugate combines the properties of the NPs with the specific and selective recognition capability of antibodies. The protection ensured by the shell extremely increases dyes photostability compared to commercial available dyes. This is a very useful characteristic in those analyses where long-time stability is required. Extending the analysis to other markers, for examples activation markers, we had some preliminary good results.

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References: J.Lumin., 117 (2006), 75-82; J.Mater. Chem.,20 (2010), 2780-2787; Nano Lett., 6 1 (2006), 84-88; Bioconjugate Techniqus, 2nd Edition, Greg T. Hermanson, Accademic Press 1996, Am. J. Clin. Pathol., 83 (4), (1985), 431-8; Differentiation, 30, 2, (1985), 130-140