Bioconjugated fluorescent silica nanoparticles for immunofluorescence assays

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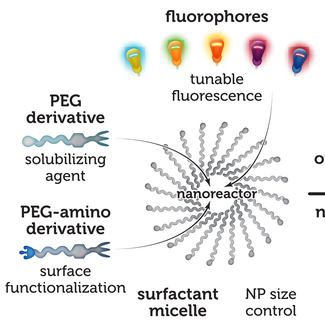
Introduction

Fluorescent antibodies are widely employed in various immunofluorescence assays, but molecular fluorophores commonly used for bioconjugations lack in photostability and the intensity of the emitted light could be pH dependent or quenched by external agents. Fluorescent silica nanoparticles help to overcome these limitations, by enclosing the fluorophores inside their protective matrix. Morevoer, silica offers many other advantages, being transparent to UV and visible light, biocompatible, non-toxic, inexpensive and extremely versatile.

Silica Nanoparticles



The synthesis of silica nanoparticles is based on the hydrolysis and condensation of an organosilane precursor inside a micelle, in order to control the size (10-200 nm) and to improve the monodispersion of the system. To obtain fluorescent and functionalized nanoparticles, other reagents – previously modified with a trialkoxysilane moiety, so that they may form covalent bonds with the silica matrix – are needed:

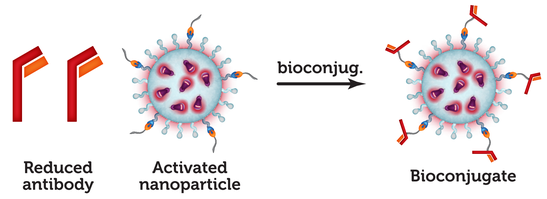
- a PEG derivative – to stabilize the nanoparticles in water;
- a PEG-amino derivative – to functionalize the surface;
- a fluorescent molecule.

Bioconjugation

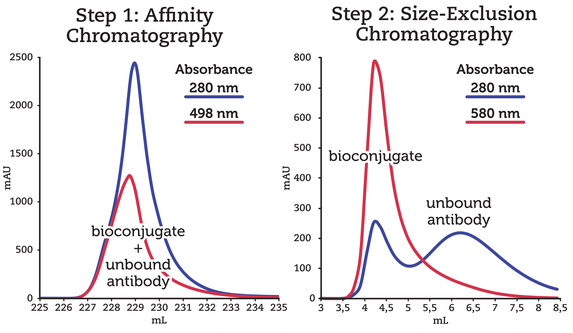
The conjugation of antibodies to nanoparticles yields a brightly fluorescent system, retaining the specific molecular recognition ability of the antibodies to antigens.

Bioconjugation Steps:

- Activation of nanoparticles with a crosslinker;
- Reduction of the antibody;
- Bioconjugation reaction;
- Purification by means of affinity and size-exclusion chromatography.



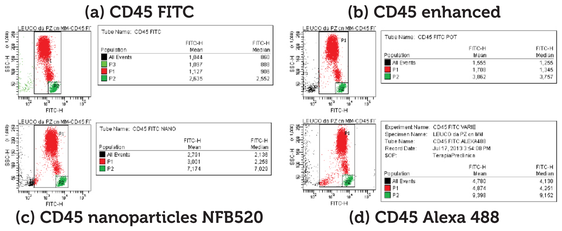
Purification Steps



Affinity chromatography eliminates unbound nanoparticles, while size-exclusion chromatography separates the unbound antibody from the bioconjugate, thus reaching a high degree of purity (96%).

Flow Citometry

CD45 FITC-LIKE



CD45 antibody conjugated to nanoparticles NFB520 (c) shows a brighter fluorescence and a better discrimination than CD45 FITC (a), or CD45 enhanced (b), but similar to the Alexa conjugate (d).

Images obtained by Dr. Fabbri (IRCCS - IRST Meldola)

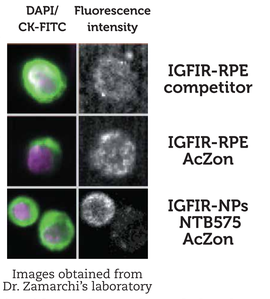
CellSearch

IGFIR RPE-LIKE

The CellSearch® system from Veridex is a test used to isolate rare cells from blood.

Cells are stained with DAPI/CK-FITC and marked with anti-IGFIR-RPE from:

- a competitor;
- a conventional conjugation in AcZon;
- the innovative conjugation with AcZon NPs RPE like.

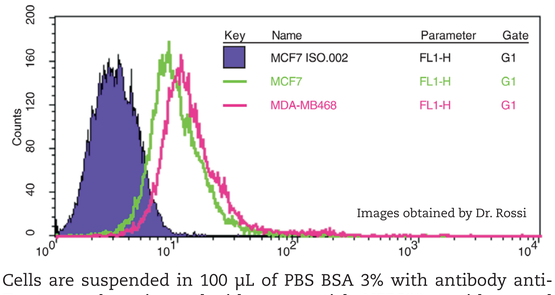


Images obtained from Dr. Zamarchi's laboratory

Nanoparticles clearly show a significantly reduced background.

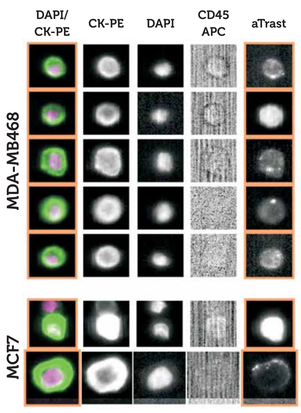
TRASTUZUMAB FITC-LIKE

MCF7 and MDA-MB468 are two breast cancer cell lines HER2/neu non-amplified, that is, they express low levels of this protein on the surface. Both cell lines were treated for 20 minutes with Herceptin® at the same dose used with patients, then washed and analyzed in flow cytometry and with the CellSearch® system.



Cells are suspended in 100 µL of PBS BSA 3% with antibody anti-Trastuzumab conjugated with nanoparticle NFB520 or with control isotypes, incubated for 20 minutes on ice in the dark, then washed, centrifuged, resuspended and analyzed in flow cytometry (FACSCalibur).

The two control isotypes are coincident, while cell lines labeled with Trastuzumab show to bind the antibody at low intensity (as expected when the antigen is not overexpressed).



200 cells are inoculated in 7,5 mL of healthy donor blood; then, the spiked sample is analyzed with the CellSearch® system, following the automated procedure used to treat patients samples, with the addition of anti-Trastuzumab, at the same dose of other integrated tests (anti-M30, anti-IGFIR). At the end of the procedure, the cartridges are scanned at various integration times and analyzed.

Images show that this labeling can highlight cells which exhibit on the membrane HER2/neu+Herceptin®, either bright or dim.