Bioconjugated fluorescent nanoparticles: an improved tool for immunofluorescence assays

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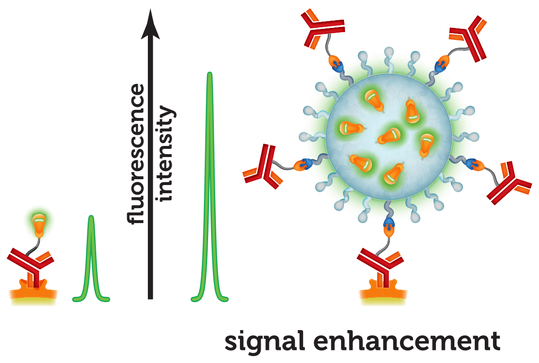
Introduction

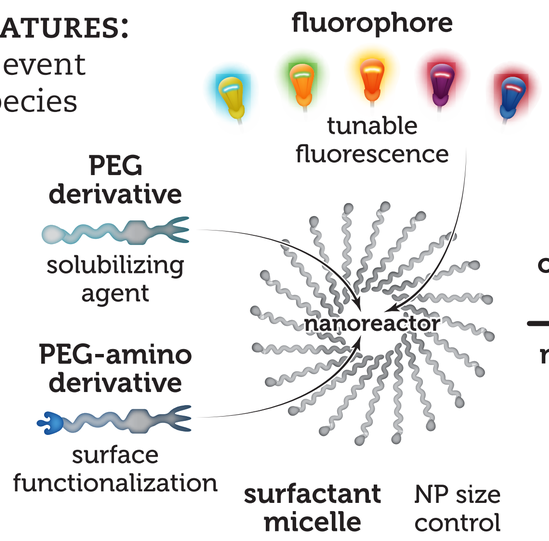
The design and production of ever more responsive analysis tools are essential for the early diagnosis and treatment of a disease. On one hand, antibodies are highly specific markers for the recognition of biomolecular targets, while on the other hand, fluorescence is a sensitive and versatile technique for detection purposes. As a matter of fact, fluorescent antibodies are widely employed in immunofluorescence assays, but molecular fluorophores commonly used in bioconjugations lack in photostability and the intensity of the emitted light may be pH dependent or quenched by external agents.

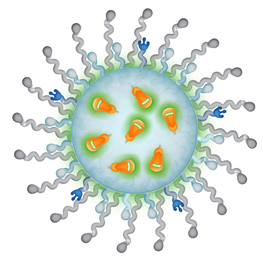
Silica Nanoparticles

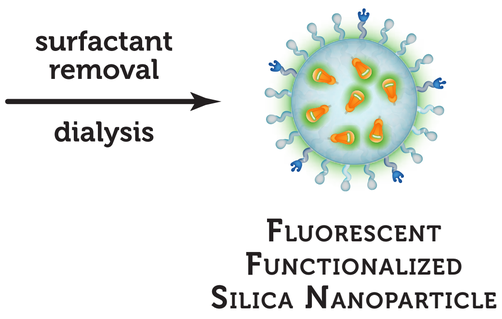
Dye-doped nanoparticles improved features:

- Many fluorophores signal the recognition event
- NPs matrix protects against interfering species
- Photobleaching is reduced







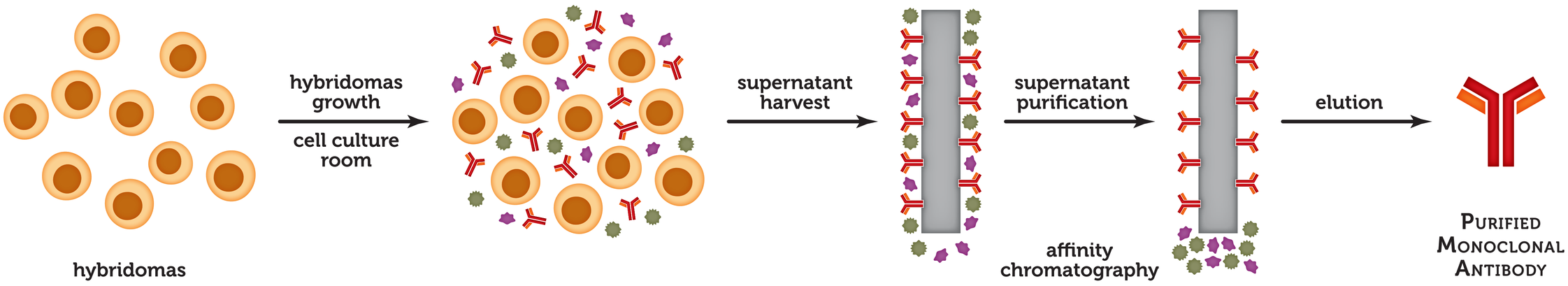


Silica advantages:

- Transparent to UV-Vis light
- Biocompatible and non-toxic
- Inexpensive
- Chemistry well-known

Monoclonal Antibodies

Hybridoma technology - Hybrid cells, from myeloma cells and B lymphocytes, release monoclonal antibodies in culture medium.
This supernatant, once harvested, is purified through affinity chromatography.
The purified antibodies are submitted to quality control, then they are ready for successive conjugation steps.



Bioconjugation

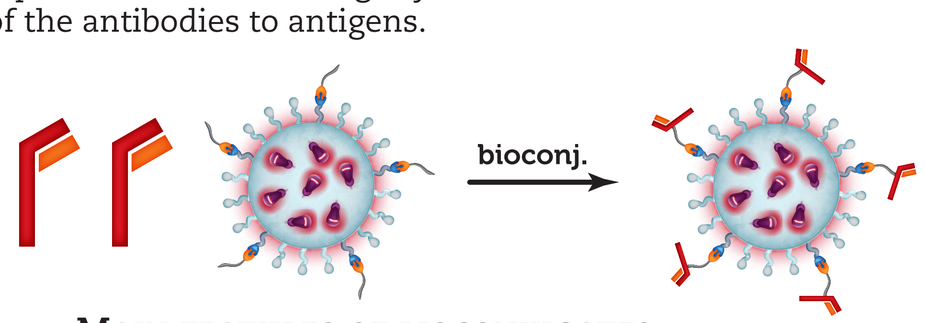
By conjugating nanoparticles with antibodies, it is possible to create a brightly fluorescent device having the specific molecular recognition ability of the antibodies to antigens.

Different methods of activation,
both for the antibody and for the nanoparticle:

- Activation of nanoparticles with SMCC
- Reduction of the antibody

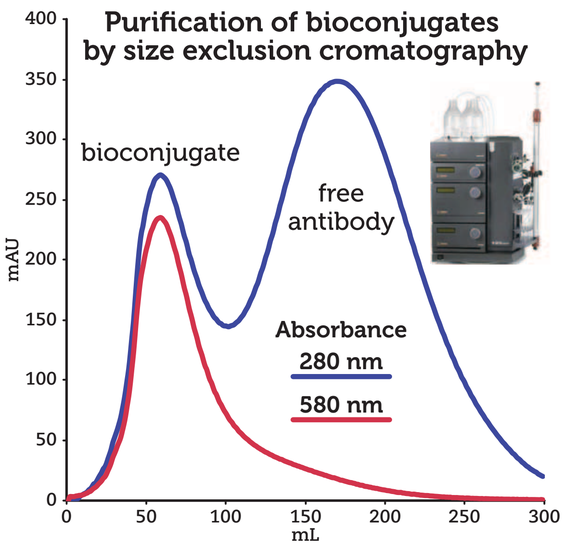
- Activation of the antibody with SMCC
- Sulfhydryl-modified nanoparticles

- Activation of the antibody with SMCC
- Activation of nanoparticles with heterobifunctional PEG derivatives



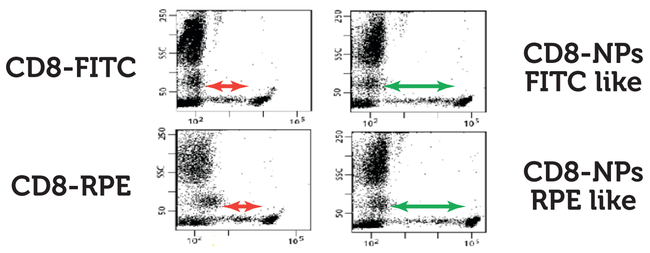
Main features of bioconjugates

- High fluorescence intensity
- High stability in aqueous solutions over time
- Great stability at different pH and ionic strength
- Compatibility with modern flow cytometers



Applications

Flow Cytometry



Dot plots comparing anti-CD8 conjugated with traditional fluorophores and with fluorescent silica nanoparticles that provide better discrimination between cellular populations.

Images obtained in AcZon on a Partec PAS II

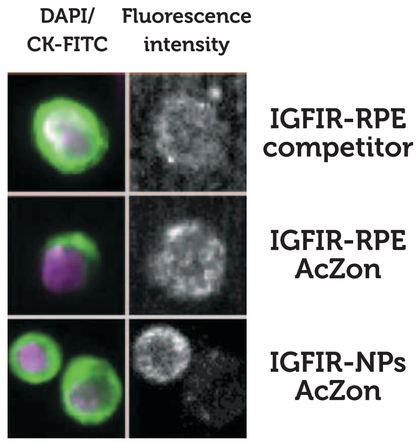
CellSearch® System

The CellSearch® system from Veridex is a blood test used for rare cells isolation.

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 nanoparticles RPE like.

Nanoparticles clearly show a significantly reduced background.



Images obtained from Dr. Zamarchi Laboratory (IOV – Padova)