A new alternative to PE-Cy5 tandem dye for flow cytometry

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Introduction

Silica nanoparticles are an interesting nanomaterial for theranostics purposes due to their unique physical and chemical properties. Fluorescent silica nanoparticles are emergent fluorescent probes for life science applications, i.e., for Flow Cytometry (FC). Here we present a new kind of core-shell fluorescent silica nanoparticle (NP) excitable with the blue laser of flow cytometer (488nm) that shows a bright emission peaked at 665nm. The fluorophores are embedded inside the silica core allowing an enhanced Stokes shift, thanks to FRET (Förster Resonance Energy Transfer) mechanism (figure 1). The shell is functionalized for further conjugations to antibodies (Ab) and/or other molecules. The Ab-NP conjugate has many advantages: it is brighter, it is more photostable than common protein/organic dye conjugates and it can be purified twice resulting in a higher purity of the final product.

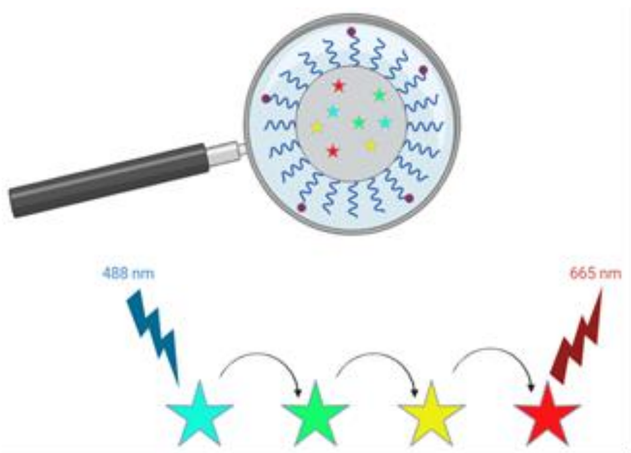

Figure 1. Scheme (simplified) of FRET mechanism inside the NP.

NP antibody conjugate synthesis and purification

The NP synthesis is made in water through a micelle-assisted process with the help of a surfactant. The reagents arrange inside the micelle so that the resulting nanoparticle has a silica core and a shell of PEG (figure 2).

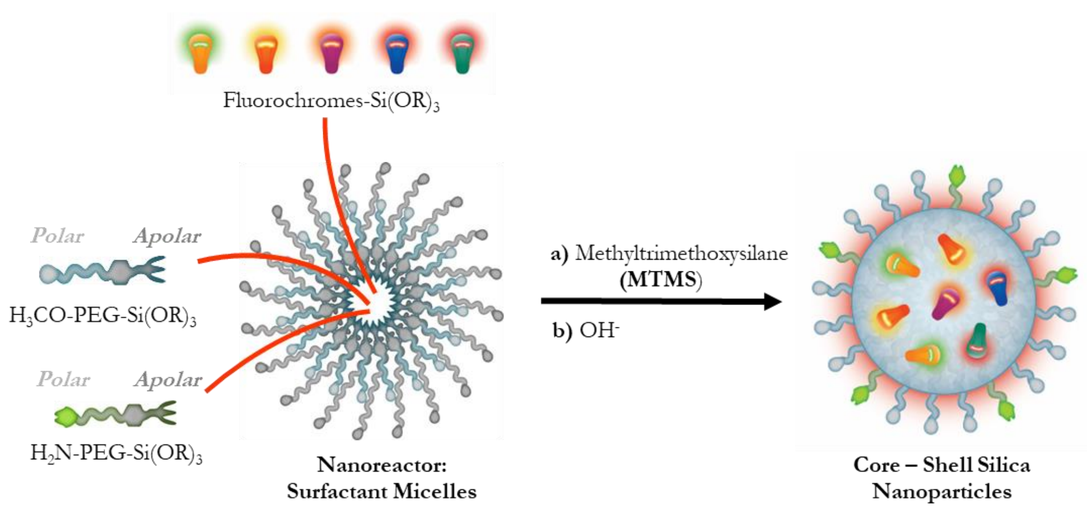

Figure 2. Scheme of NP synthesis.

Fluorophore-silanes are covalently bound inside the core, while the addition of silane-PEG-NH_2 results in NP shell functionalization for further conjugation thanks to the activation with a linker. The Ab-NP conjugate is purified twice by size exclusion chromatography (SEC) and affinity chromatography (AC) The result is a high purity conjugate.

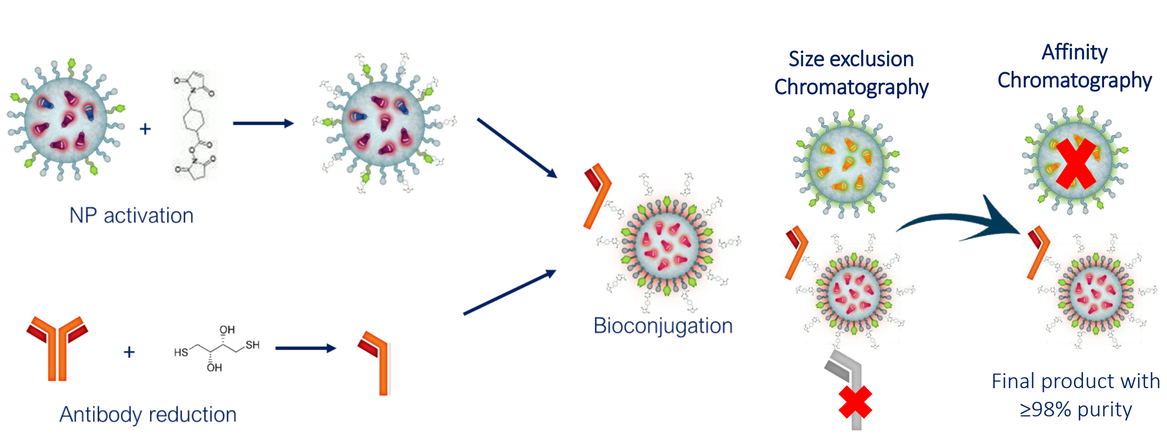

Figure 3. Scheme of NP-Ab conjugate synthesis and purification process.

Results

Mouse anti-Human CD8-NT_B660 (PE-Cy5 like) conjugate was tested in flow cytometry on fresh PBMCs (peripherical blood mononuclear cells) from healthy donors and compared to a golden standard. The results (table 1 and figure 4) showed that, compared to traditional PE-Cy5 conjugate, NT_B660 one provides a better discrimination of the positive population (CD8+). Thanks to the improved discrimination, NP-conjugate highlights also the dim population of CD8+ cells, as visible inside the blue circle on the right figure 5.

		Mean +	CV% +	Mean -	CV% -
Anti CD8	conjugated to **Pe-Cy5**	1315	43,37%	33084	39,40%
	conjugated to **NT_B660**	3321	64,03%	126434	40,61%

Table 1. Results of flow cytometry experiments with ant CD8 conjugates.

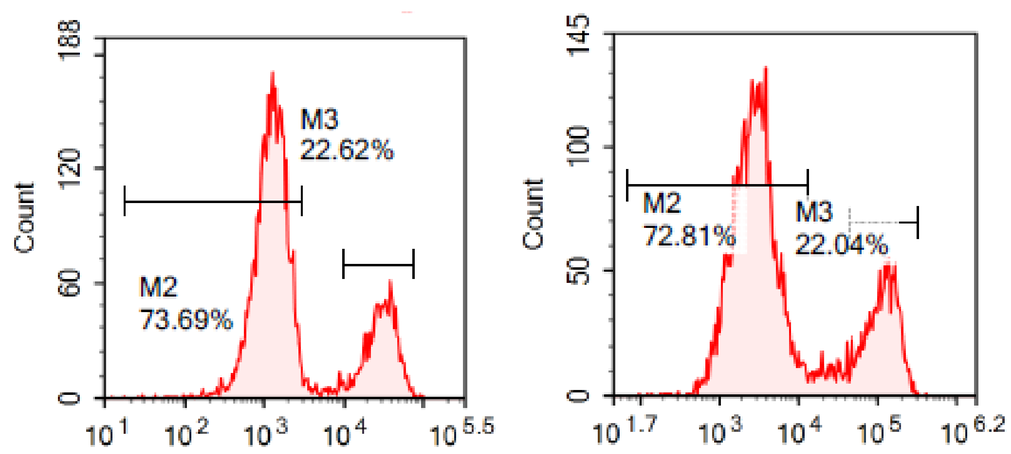

Figure 4. Left histogram for anti CD8-PE-Cy5 golden standard. Right histogram for anti CD8-NT_B660.
Flow Cytometer: NovoCyte 3000 (Acea - Agilent Technologies)
Software for analisys: NovoExpress version 1.2.5

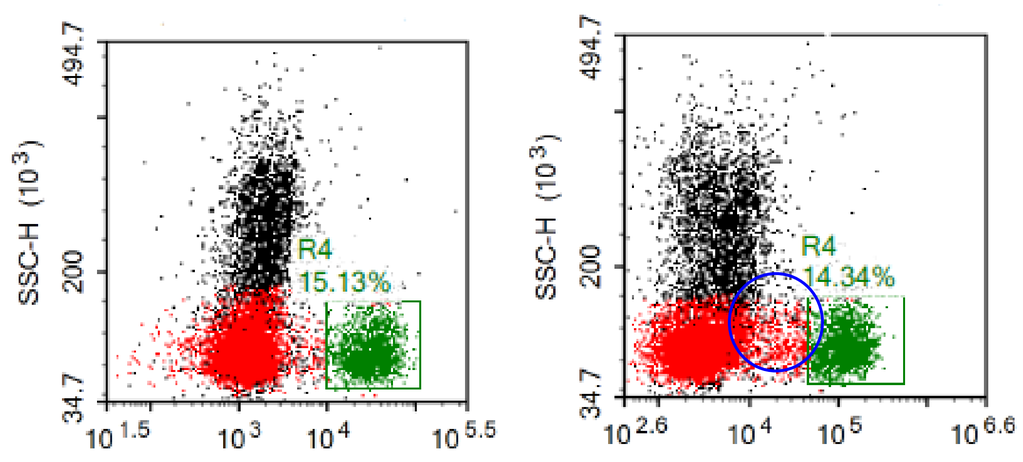

Figure 5. Left dot-plot for antiCD8-PE-Cy5. Right dot plot for anti CD8-NT_B660, in the blue circle the dim population.

Future Perspectives

Further increasing of the FRET efficiency, by replacing one or more fluorophores, could boost the brightness of nanoparticles and resulting conjugates. Here we showed the absorption and emission spectra of a new type of NP currently under development (figure 6). The emission at λ<620nm is decreased with respect to other tandem dyes, like PE-Cy5 (data not shown), allowing the contemporary employment of different probes emitting at λ<620nm in the same sample. In addition, the brightness is increased leading to an enhanced discrimination of the positive population.

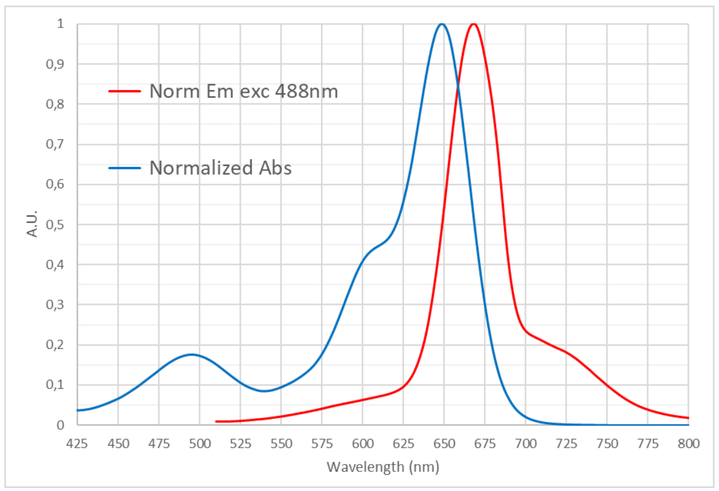

Figure 6. Normalized Absorption and emission spectra