Synthetic optimization of core-shell silica nanoparticles with tunable fluorescent characteristic: a novel probe for flow-cytometry.

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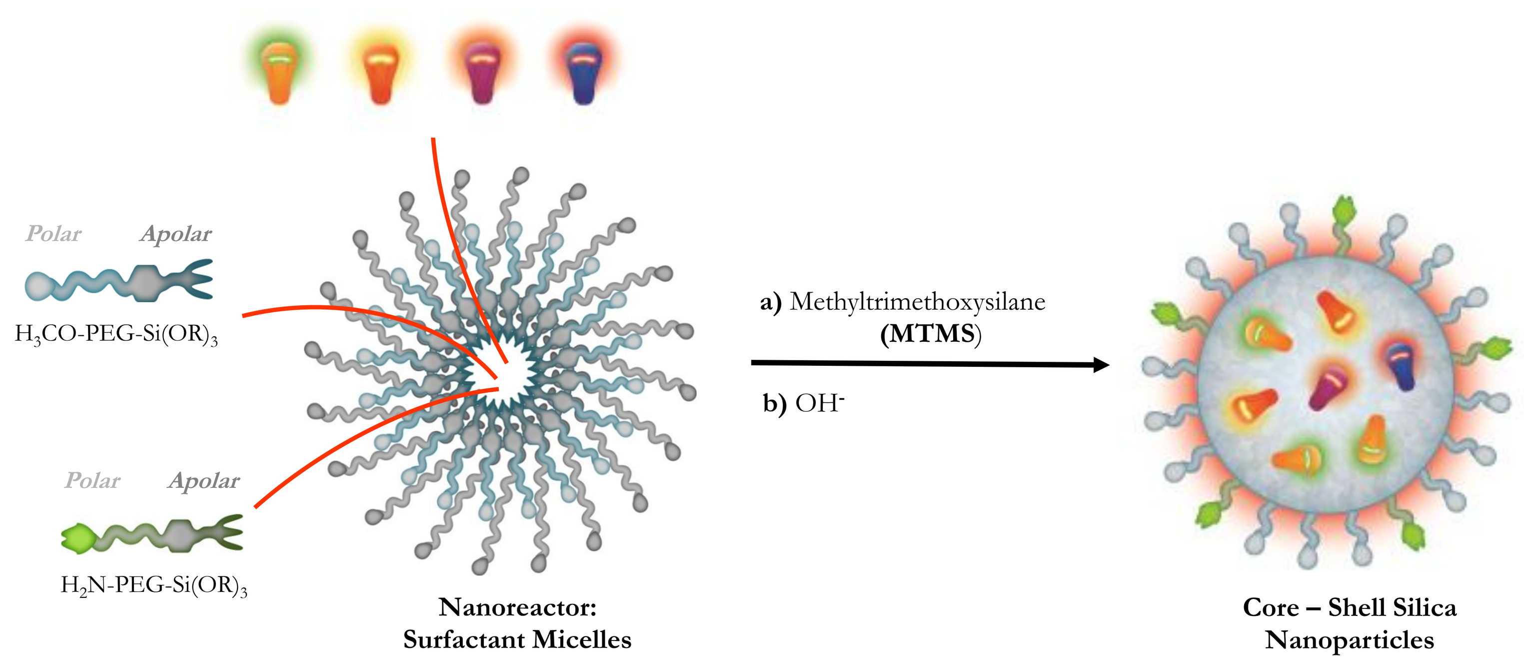
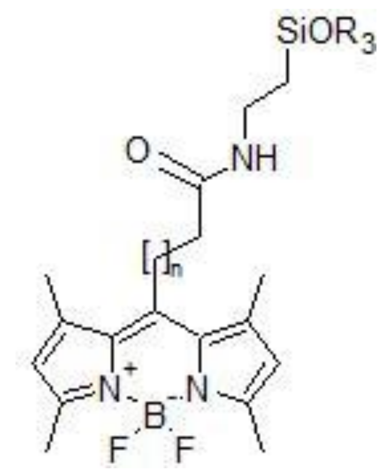
Nano Bio Tech

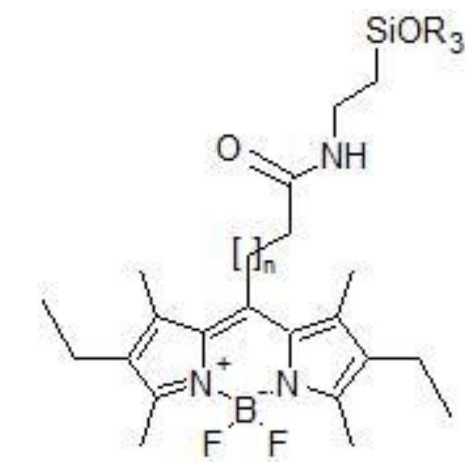
Aczon Nanoprobes are **core-shell dye doped silica nanoparticles** synthesized through a micelle-assisted method, where a surfactant is used to create a nanoreactor within which reagents arrange. The base-catalysed hydrolysis of a silane precursor along with different dyes, functionalized with a trialkoxysilane group able to covalently link to the silica matrix, leads to the formation of fluorescent monodisperse nanoparticles.

To obtain nanoparticles excitable with blue laser and that emit in the near-IR (**NT_B660)**, four different dyes were selected to be simultaneously entrapped into silica core. The strong interconnection reached inside the core should lead to Florescence Resonance Energy Transfer (**FRET**).

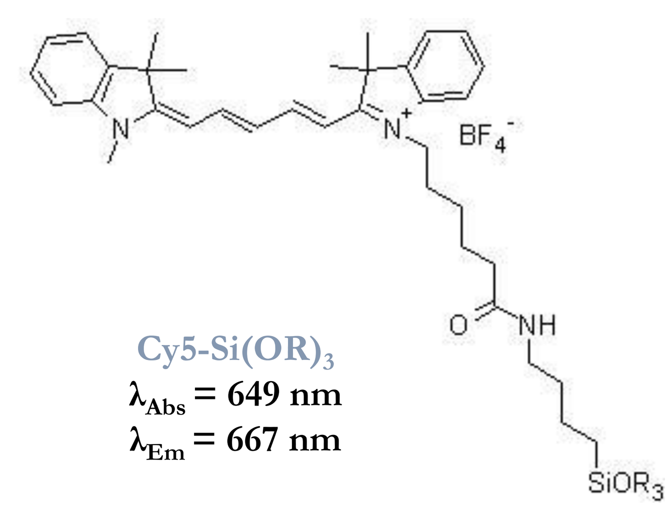




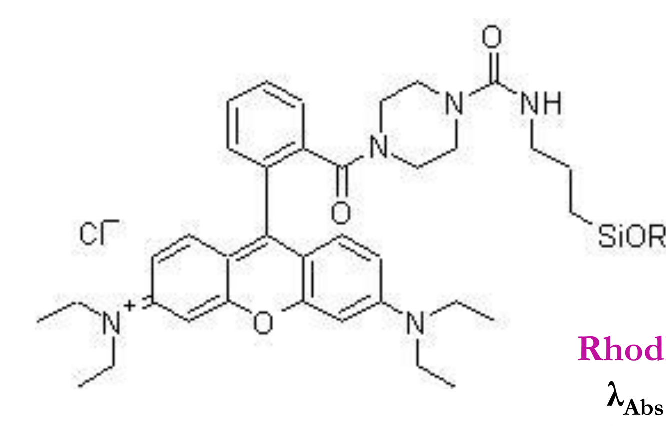
TMB-$Si(OR)_3$
λ_{Abs} = 498 nm
λ_{Em} = 506 nm



DEB-$Si(OR)_3$
λ_{Abs} = 521 nm
λ_{Em} = 531 nm



Cy5-$Si(OR)_3$
λ_{Abs} = 649 nm
λ_{Em} = 667 nm



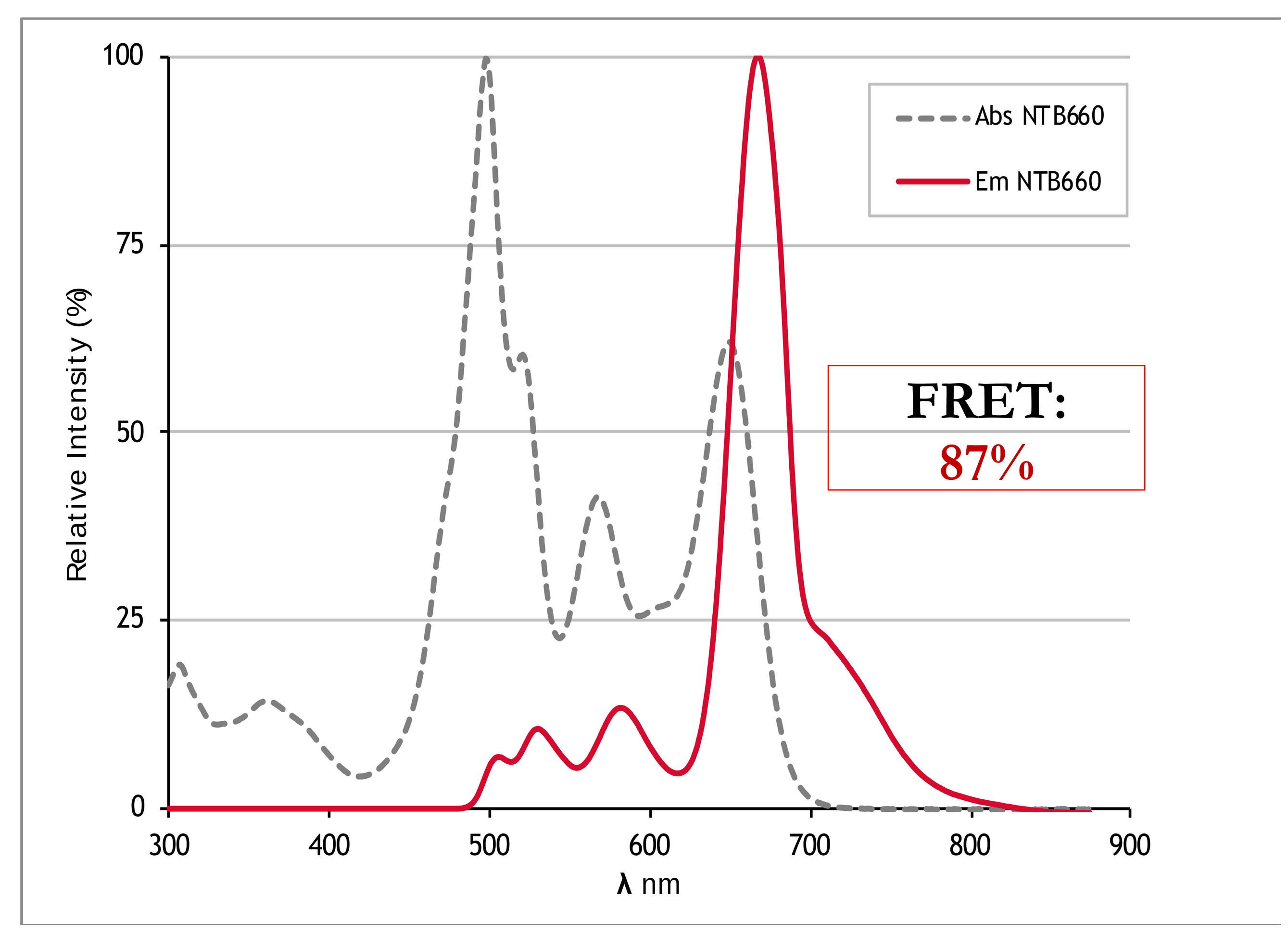
RhodBP-$Si(OR)_3$
λ_{Abs} = 566 nm
λ_{Em} = 586 nm

The two main factors that influence dimensions and monodispersion of these nanoparticles are the nature of the silane precursor and surfactant. To find the optimal reaction conditions, we used a trial and error approach. Changing the silane precursor has not proven to be a useful strategy. Otherwise, the modification of the type of surfactant, allowed us to reach **87%** of **FRET efficiency**.

Surfactant variation

Surfactant	Organo-Silane Precursor	FRET
Triton X-100	Methyltrimethoxysilane (MTMS)	NO
Nonidet	MTMS	70%
Tween 20	MTMS	NO
Brij58	MTMS	73%
C12E10	MTMS	NO
Brij L4	MTMS	NO

- Surfactant concentration
- Temperature
- Base concentration
- Silane precursor concentration



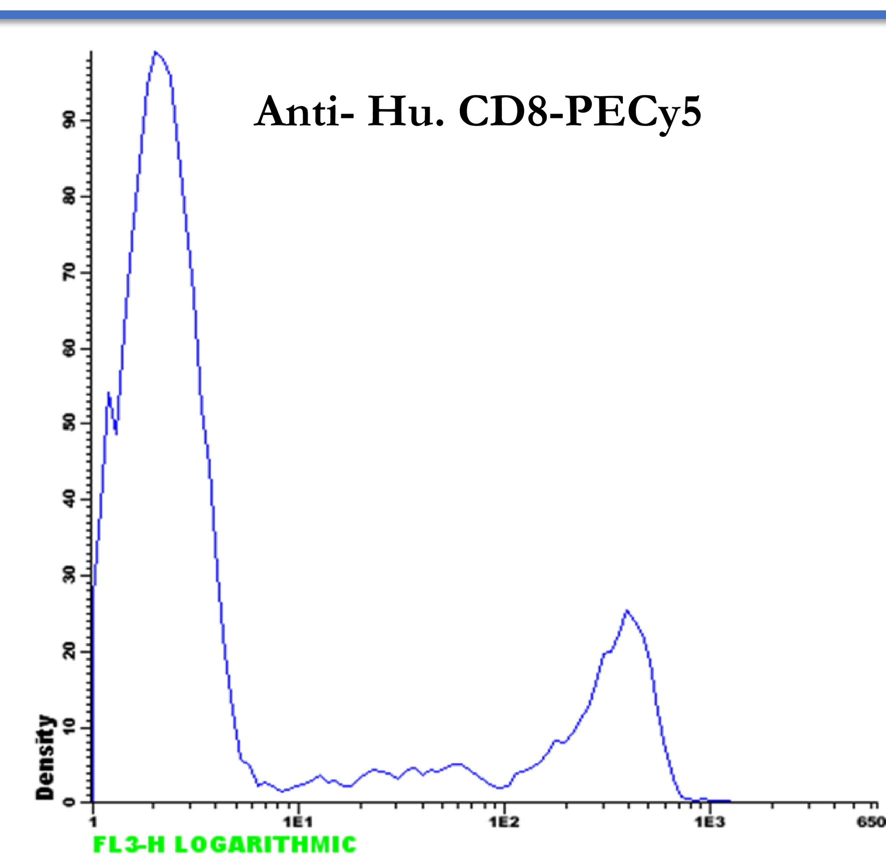
FRET efficiency has been calculated considering the emission intensity of the final acceptor and average intensity of donors.

To prove the ability of those Nanoprobes to compete with commercial dyes, nanoparticles were conjugated with mouse anti-Hu. CD8 (733; Ms IgG1) antibody exploiting amine reactive groups on the nanoparticle surface.

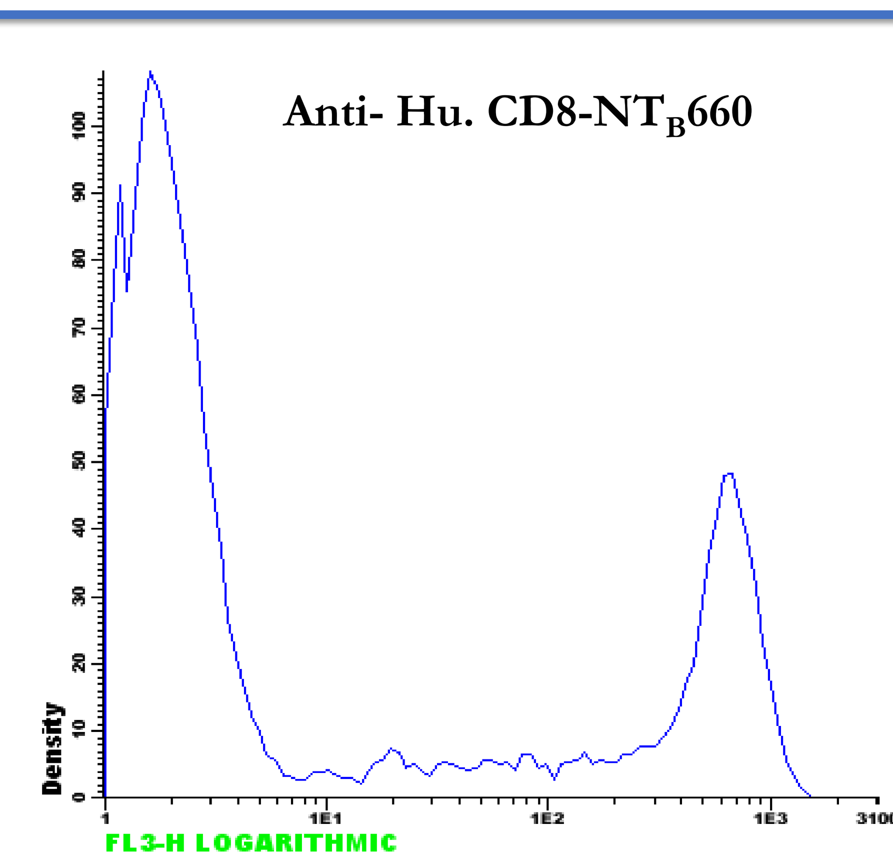
Cytograms compare anti-Hu. CD8-NT_B660 against the classical fluorophores anti-Hu. CD8-PECy5 on BD FACScan flow-cytometer.

Emission intensity (positive mean), as well as the signal to back-ground ratio, of **NT_B600** is higher than commercial dyes. Results clearly demonstrate not only the good photo-chemical characteristics of our Nanoprobes but also the conjugation efficiency.

Anti- Hu. CD8-PECy5



Anti- Hu. CD8-NT_B660



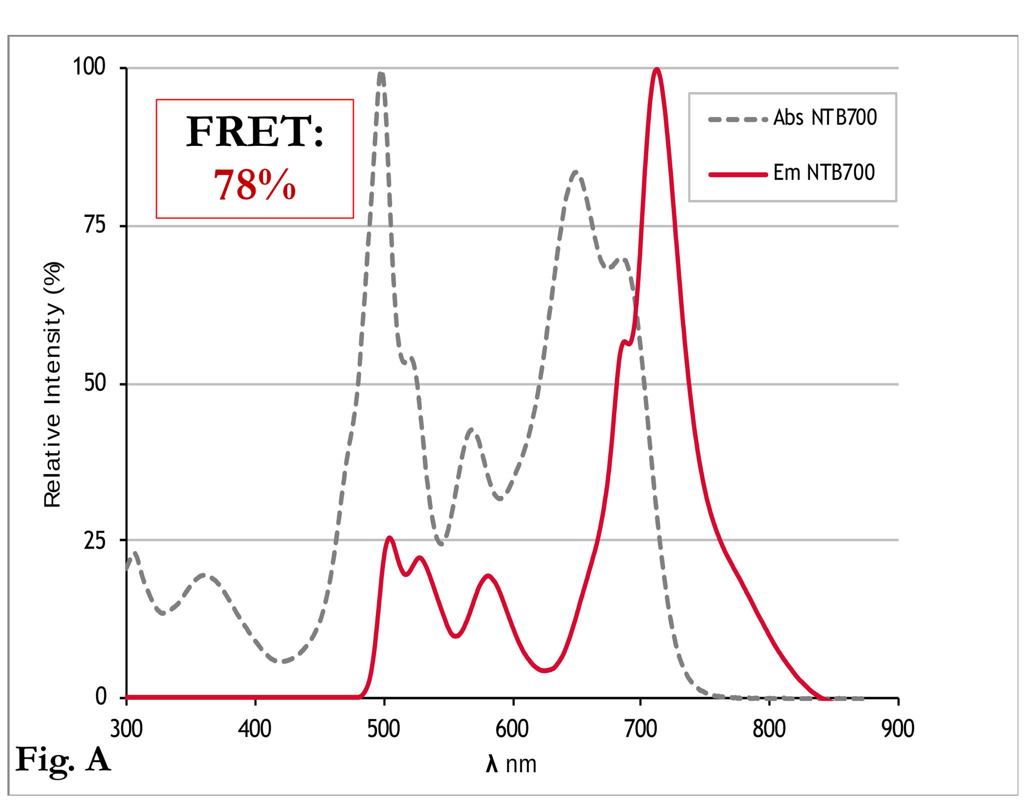


Fig. A

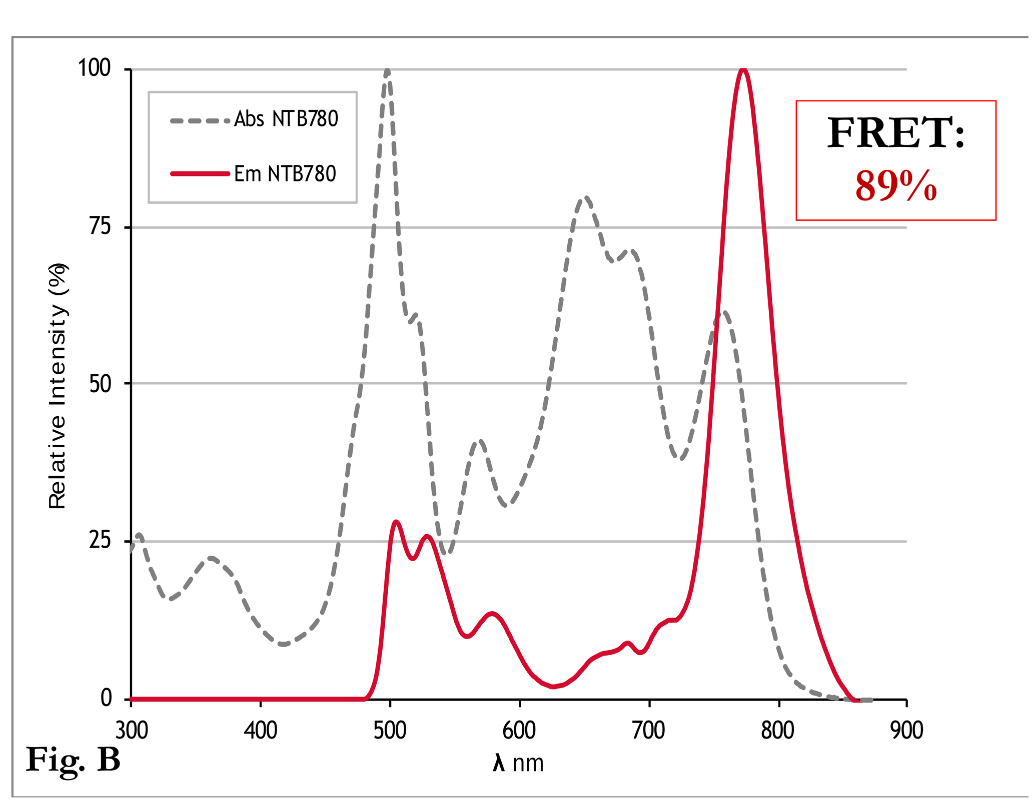


Fig. B

We have also conducted preliminary experiments targeted to modulate nanoparticles photochemical characteristics by increasing the number of entrapped fluorophores.

NT_B700 (Fig. A) contains one more dyes than NT_B660 those increasing the emission to **710 nm**.

NT_B780 (Fig. B) contains six different dyes and emits at **780 nm**.

REFERENCES: ***J. Mater. Chem.,*** 20 (2010), 2780-2787; ***Nano Lett.,*** 6, 1 (2006), 84-88; ***J. Lumin.,*** 117 (2006), 75-82; ***Org. Lett.,*** 5, 18 (2003), 3245-3248; ***J. Phys. Chem.***, 113 (2009), 439-447; ***Chem. Mater.***, 24 (2014), 361-372; ***Nano Lett.***, 5, 1 (2005), 113-117