Multiple dye doped core-shell silica nanoparticles: outstanding stability and signal intensity exploiting FRET phenomenon for biomedical applications.

C. Pellegrino, A. Volpe, R. Juris, M. Menna, V. Calabrese, F. Sola, C. Barattini, A. Ventola

Aczon S.r.L.- *Via Lavino 265/D- 40050- Monte San Pietro (BO)- Italy*

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 are emerging as promising probes for biological application. In particular, during the last decades, fluorescent silica nanoparticles (NPs), have been extensively studied thanks to their interesting characteristics. First of all, synthesis of fluorescent core-shell silica nanoparticles ensure the dyes solubility in water. Moreover, these biocompatible systems provides the dual purpose of increasing fluorescence intensity and photostability. Multiple dyes are entrapped into each nanoparticles therefore molar extinction coefficient of fluorescent silica NPs results to be higher than those of common dyes. Moreover, the protection ensured by silica core or, eventually, by an outer shell, should limit photobleaching. The flexibility of dye doped silica nanostructures opens up plenty of possibility to gain switchable and responsive signal by only changing the time and numbers of fluorochromes entrapped into the core.

AIM

Here we present a one pot synthesis of core-shell silica nanoparticles doped with different numbers of dyes to ensure high efficiency Fluorescence Resonance Energy Transfer (FRET). Dyes are individually entrapped into silica core without a covalent bonding between each other. Thanks to the strong interconnection achieved inside the core a FRET with efficiency up to 86% was obtained. Nanoparticles called **NT_B530**, **NT_B575** and **NT_B660** respectively contain two, three and four different dyes. Nanoparticles can be excited with a common blue laser and are characterized by a long Stoke Shift up to the near-IR emission. Photostability was tested under continuous irradiation with Mercury lamp. Specificity for flow cytometry was tested conjugating Nanoparticles with Anti-Human CD8 antibody.

SYNTHESIS

Aczon Nanoprobes are core-shell dye doped silica nanoparticles synthesized through a micelles-assisted method. The base-catalysed hydrolysis of different trialkoxy silane leads to the formation of monodisperse nanoparticles with modular dimension (20-100 nm) depending on surfactant characteristics. Surfactant is used to create a nanoreactor within which all reagents arrange.

Shell is composed by two different polyethylene glycols, both terminating with a trialkoxy silane:

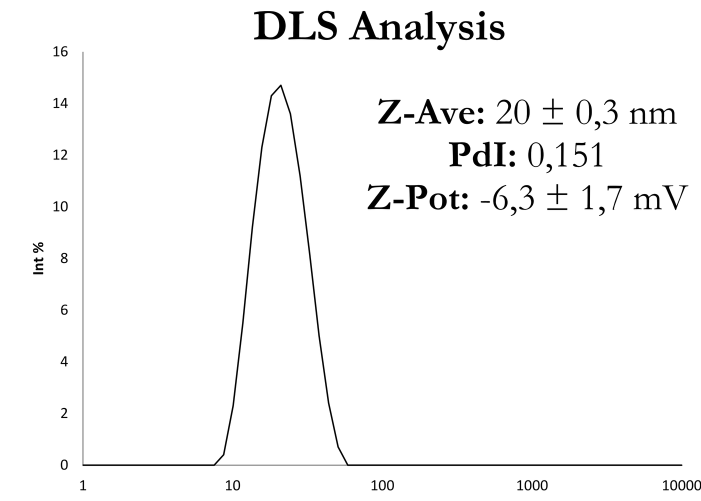
- H_3CO-PEG-$Si(OR)_3$: main component of the shell. Induces stability and solubility in water.
- H_2N-PEG-$Si(OR)_3$: introduced in a defined amount. Functionalized in order to have amine reactive groups on the external shell.

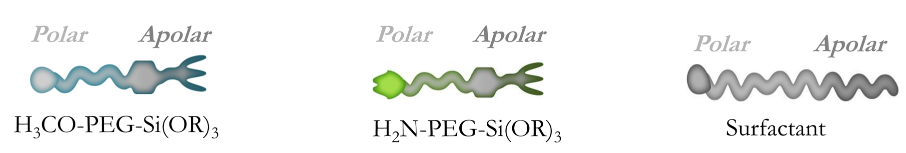
One-pot synthesis:
- Nanoreactor formation
- Addition of trialkoxy silane dyes
- Addition of organo-silane precursor
- Base catalyzed hydrolysis

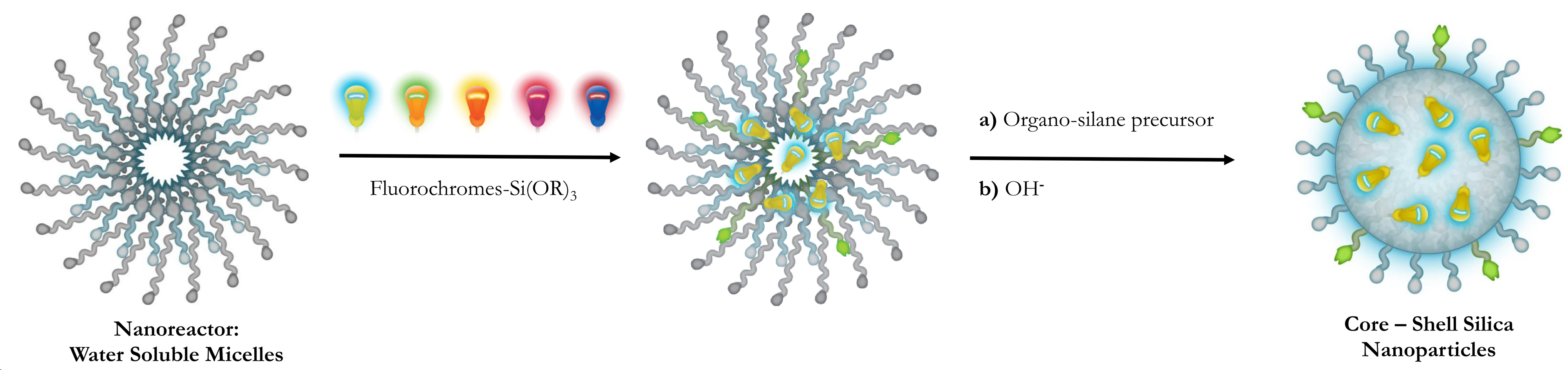
Easy Purification:
- Surfactant removal by adsorbent beads
- Dialysis
- Centrifugation

DLS Analysis

Z-Ave: 20 ± 0,3 nm
PdI: 0,151
Z-Pot: -6,3 ± 1,7 mV



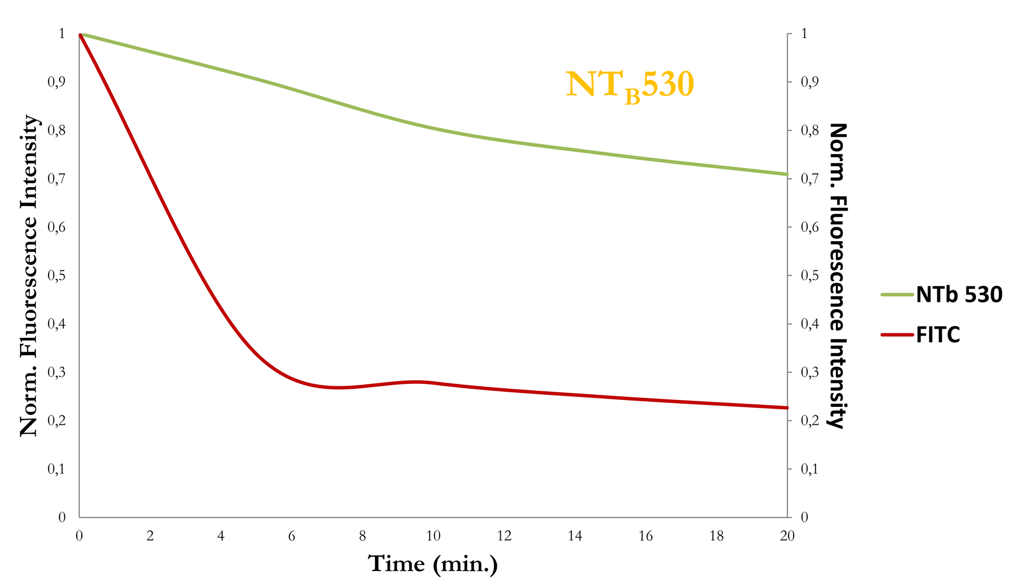




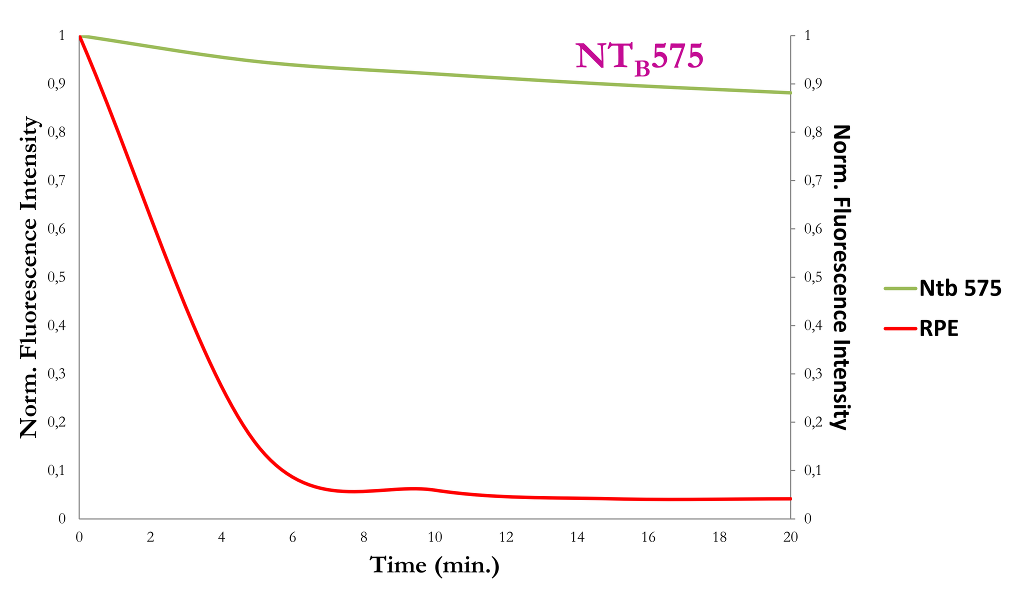
PHOTOSTABILITY

Photostability was tested entrapping nanoparticles into polyacrylamide gel matrix.

- Samples were continuously irradiated with a fluorescent mercury lamp for 20 min.
- Images were captured every 5 min.
- Images were analyzed throw Image J.
- All fluorescence intensities were normalized by the initial fluorescent intensity.



NT_B530 loses 30% of its fluorescence compared to 80% of Fluorescein (FITC)

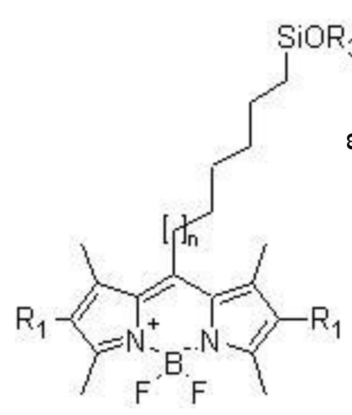


NT_B575 loses 12% of its fluorescence compared to >99% of R-Phycoerythrin (RPE)

FRET

Dyes are themselves components of silica core, thanks to a covalent modification with trialkoxy silane group.
High core hydrophobicity allows the use of non-water soluble dyes whose quantum yield is higher inside nanoparticles.

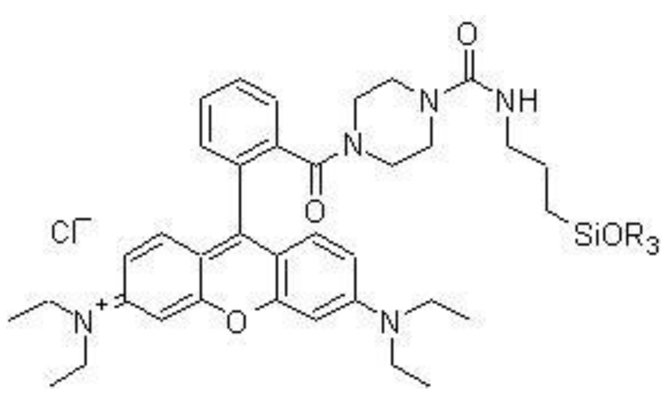
- **NT_B530** → two Bodipy-like dyes known as **TMB-$Si(OR)_3$** and **DEB-$Si(OR)_3$**.
- **NT_B575** → **TMB-$Si(OR)_3$** and **DEB-$Si(OR)_3$** together with a Rhodamine-like structure known as **RhodBP-$Si(OR)_3$**.
- **NT_B660** → **TMB-$Si(OR)_3$**, **DEB-$Si(OR)_3$**, **RhodBP-$Si(OR)_3$** together with a Cyanine-like structure known as **Cy5-$Si(OR)_3$**.



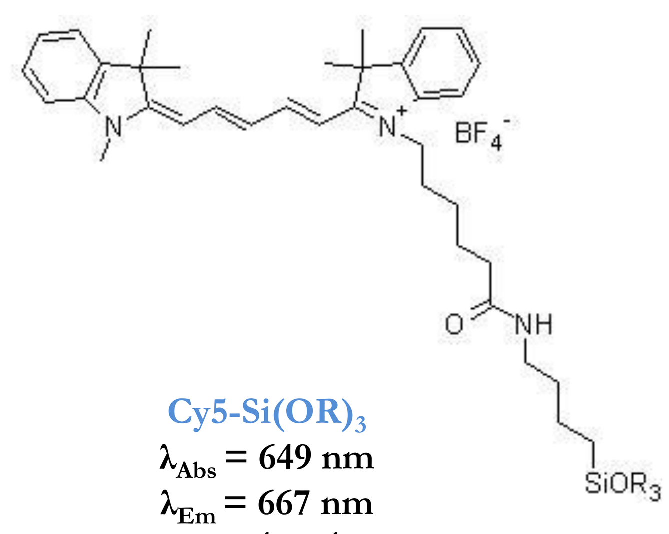
R1= H, TMB-$Si(OR)_3$
R1= Et, DEB-$Si(OR)_3$

TMB-$Si(OR)_3$
λ_{Abs} = 498 nm
λ_{Em} = 506 nm
ε = 56800 $M^{-1}cm^{-1}$ (in NPs)
Φ = 0,63 (in NPs)

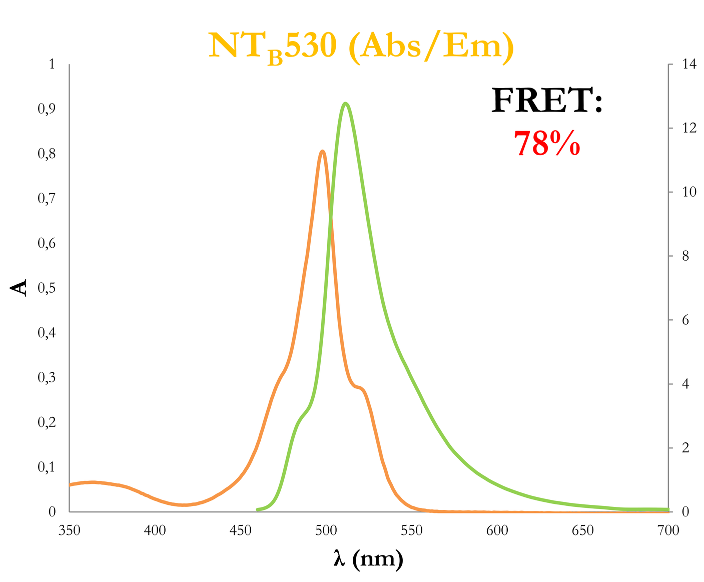
DEB-$Si(OR)_3$
λ_{Abs} = 521 nm
λ_{Em} = 531 nm
ε = 48200 $M^{-1}cm^{-1}$ (in NPs)
Φ = 0,51 (in NPs)

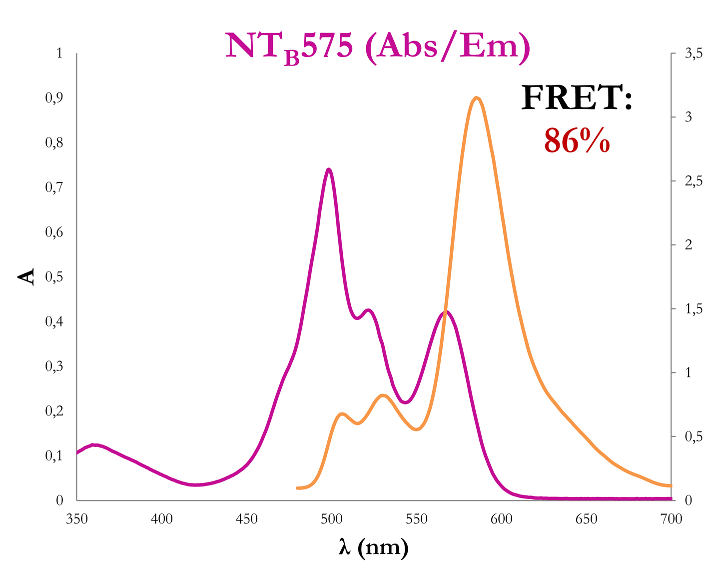


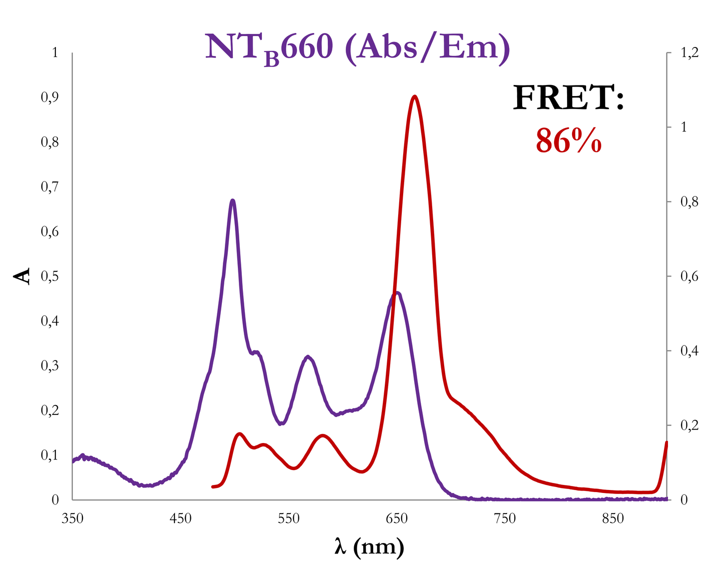
RhodBP-$Si(OR)_3$
λ_{Abs} = 566 nm
λ_{Em} = 586 nm
ε = 45200M $^{-1}cm^{-1}$ (in NPs)
Φ = 0,33 (in NPs)



Cy5-$Si(OR)_3$
λ_{Abs} = 649 nm
λ_{Em} = 667 nm
ε = 130500 $M^{-1}cm^{-1}$ (in NPs)
Φ = 0,15 (in NPs)



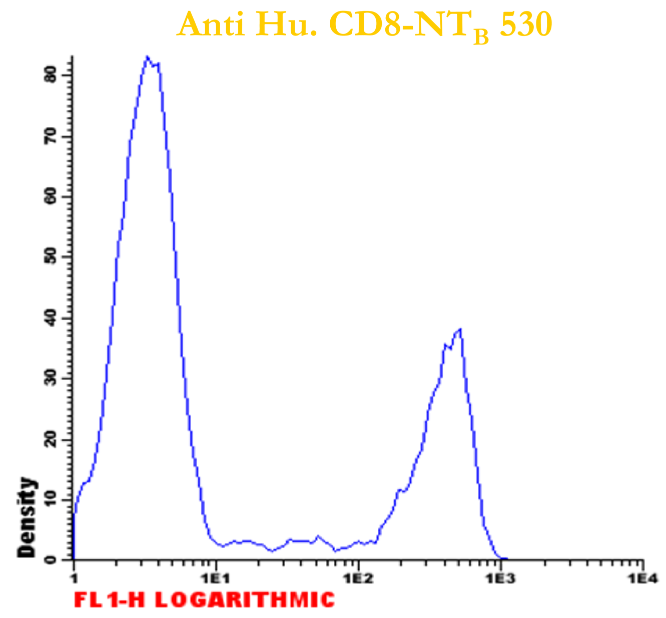


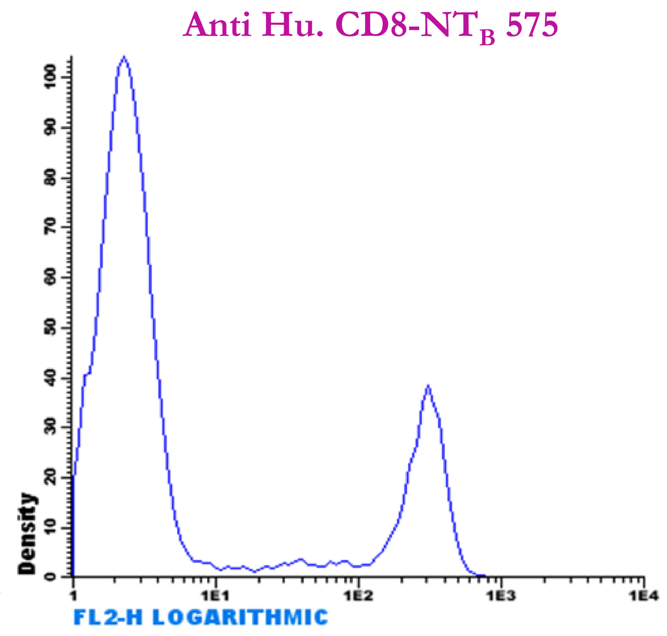


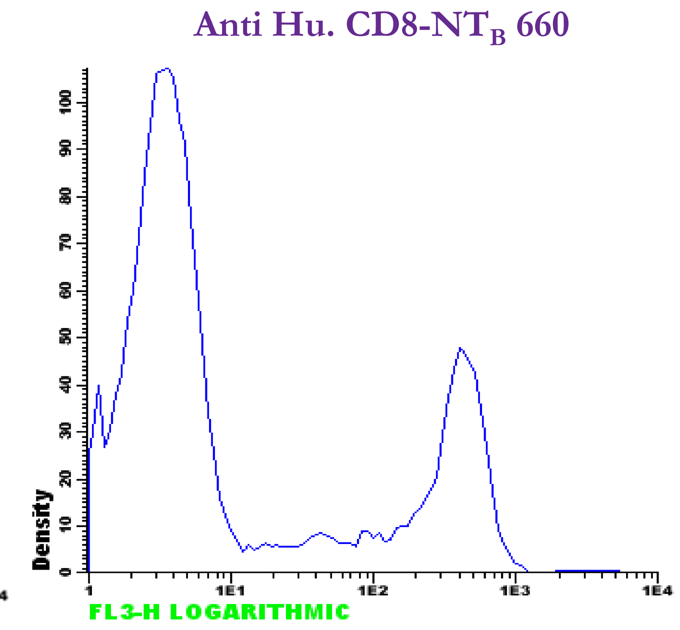
FRET efficiency has been calculated considering the emission intensity of the final acceptor and the one of the first donor.

FLOW CYTOMETRY

- NPs were conjugated with Mouse Anti-Human CD8 (733; Ms. IgG1) antibodies: **SIZE SPECIFIC CONJUGATION METHOD**
- Conjugates showed high fluorescence intensity.
- **NT_B530 two times brighter than FITC**
- Low spectral overlap in adjacent channels.







CONCLUSIONS

We efficiently synthetized monodisperse core-shell silica nanoparticles thanks to a one-pot reaction known as micelles-assisted method. Reaction characteristics allow us to obtain nanoparticles simultaneously doped with multiple dyes. The advantageous distance between dye molecules, reached inside the core, induced good Fluorescence Resonance Energy Transfer. Therefore multiple doped nanoparticles gives rise to blue laser excitable probes characterized by a long Stokes Shift up to the near-IR. Moreover, the protection ensured by the shell extremely increased dyes photostability compared to commercial available dyes.
The presence of amino groups on the surface give us the possibility to conjugate Nanoparticles with monoclonal antibodies. Flow cytometric analysis demonstrates good fluorescence intensity and low spectral overlap of the conjugates. All the aforementioned features make Aczon Nanoparticles interesting probes for both microscopy and flow cytometry.

FURTHER INFORMATION

If you are interested in more information or you want to try these tools, don't hesitate to contact us.

Tel. +39 051/6759711
Fax +39 051/6759799
www.aczonpharma.com
chiarapellegrino@aczonpharma.com

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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