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Fluorescent silica nanoparticles to improve (and monitor) targeted drug delivery

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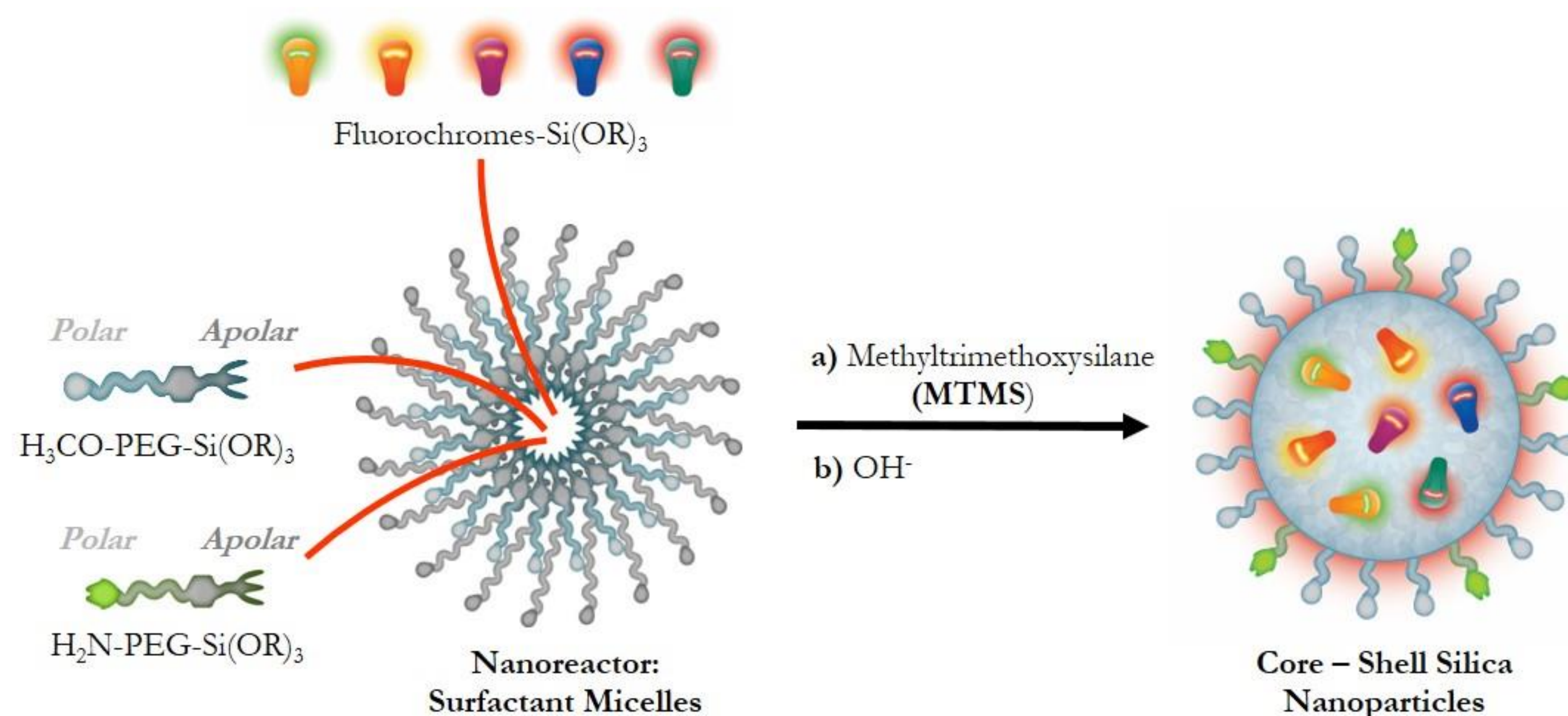
Introduction

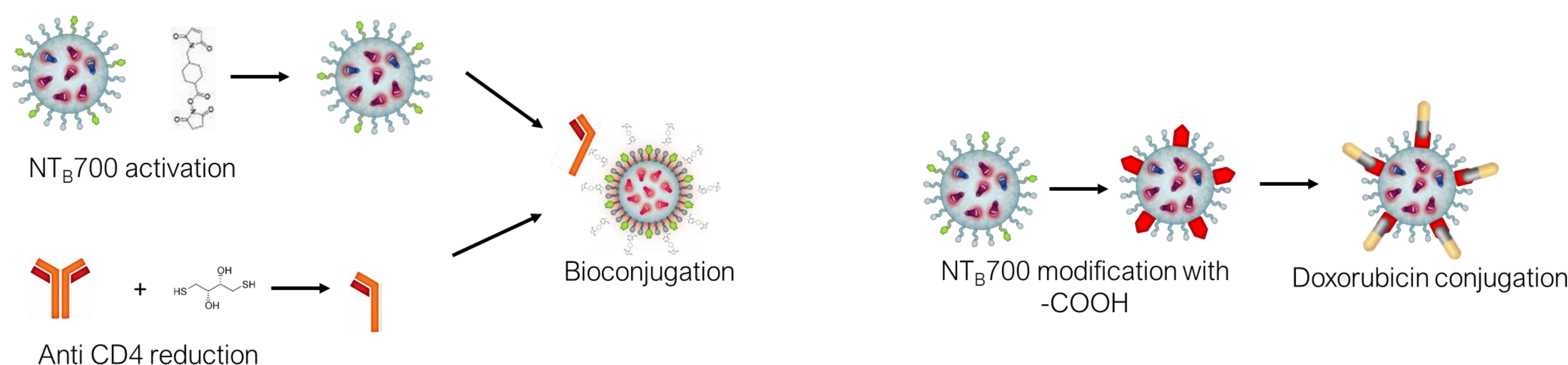
Discovery innovative treatments for cancer is, since a long time, a major problem. While chemotherapy works through different mechanisms, which includes broadly killing vigorously growing cells, including tumor but also normal cells, causing serious side effects including bone marrow suppression, hair loss, and gastrointestinal disorders. Consequently, developing drugs which more accurately target tumor cells has been one of the major focuses of cancer research in the past years. In this study we evaluate fluorescence silica nanoparticles (SiNPs) for their conjugability to both targeting molecules and drugs.

Materials and Methods

The synthesis is a modification of the Stober method called micelle assisted method: a surfactant is added to the reaction mixture to form micelles acting as nanoreactors. All the reagents, fluorophores included, conveniently modified by the addition of silane groups, arrange, spontaneously, inside the micelle due to their hydrophobicity. Ammonia and the organosilane precursor are added to the mixture and the hydrolysis and condensation reaction takes place at controlled temperature. Once the reaction was over the unreacted species were removed by dialysis, centrifugation and sequential filtrations. The result are core shell silica nanoparticles featured by a silica matrix, hosting fluorescent molecules, and a polyethylenglycole shell which can be functionalized with amine groups to be bioconjugated with drugs and/or targeting biomolecules.

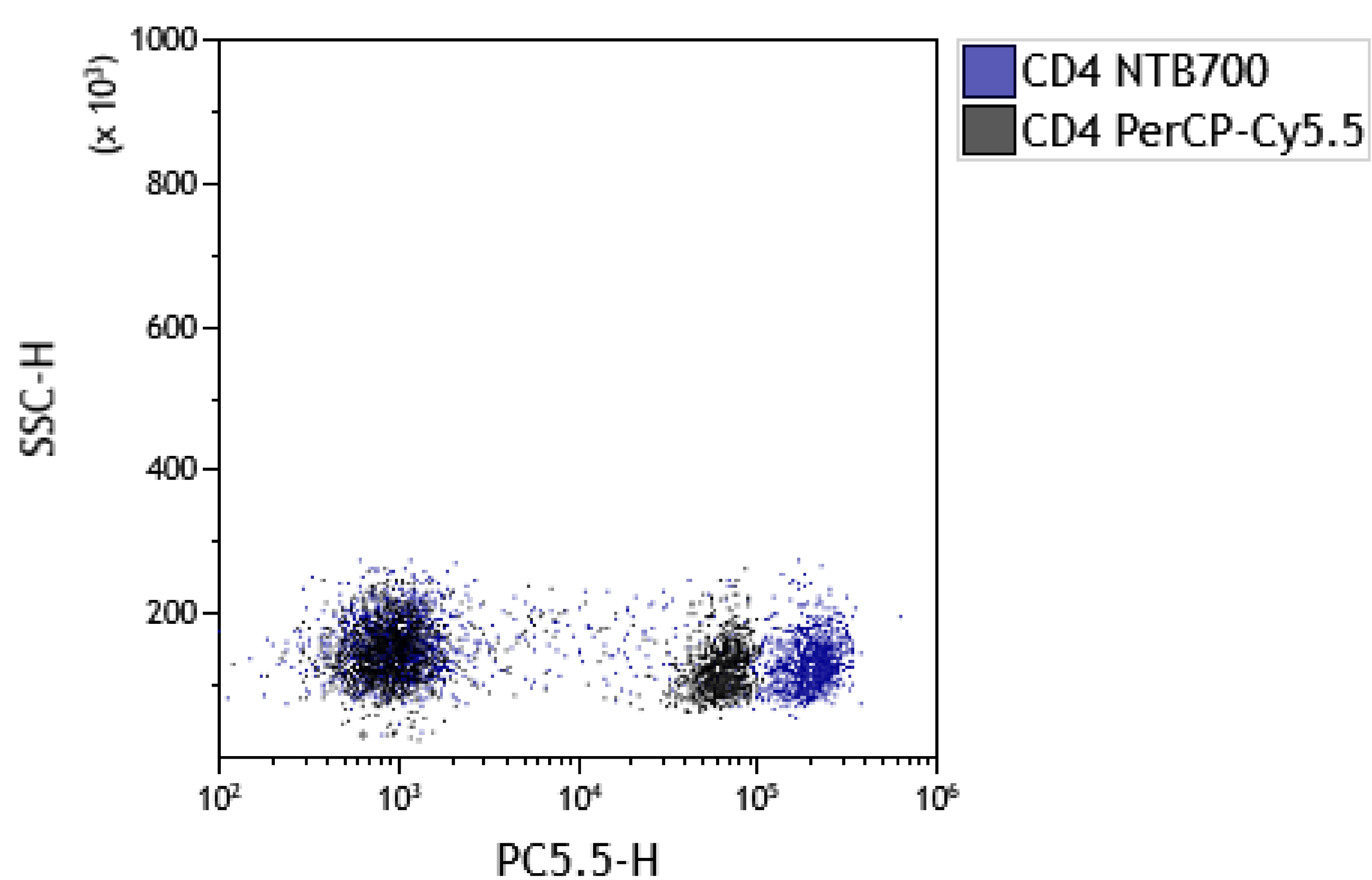


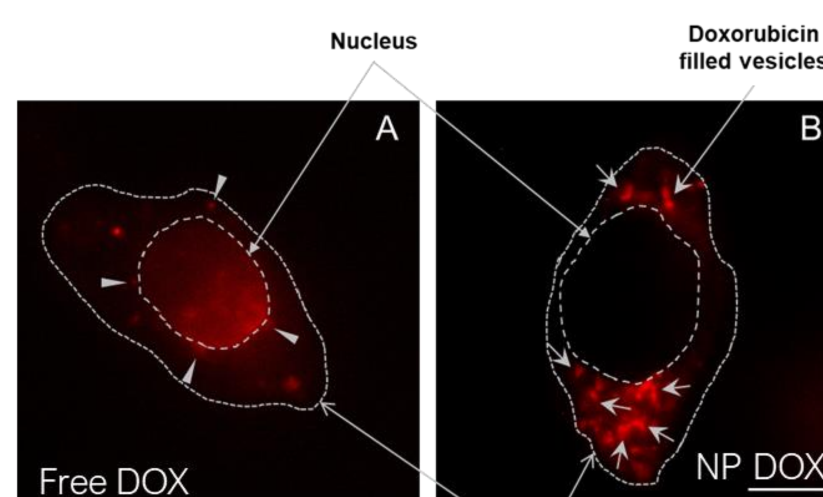
The bioconjugation protocol of SiNPs to antibodies (anti Hu. CD4) is based on the previous activation of SiNPs (NT$_B$700) with a specific cross-linker. Then, the antibody is reduced: these biomolecules contains disulfide bonds that can be reduced in order to obtain free and reactive sulfhydryl groups. Afterward, the two reactive species are mixed in a well-defined molar ratio (depending on the nanoparticles size and on the specific cross-linker employed) in which activated nanoparticles and reduced antibody were mixed. The mixture is then purified exploiting a double-step purification protocol including a preliminary size exclusion chromatography (to eliminate free antibodies) and an affinity chromatography (to eliminate unreacted SiNPs).
The conjugation of SiNPs to doxorubicin involves the preliminary transformation of the amine groups to carboxylic ones.

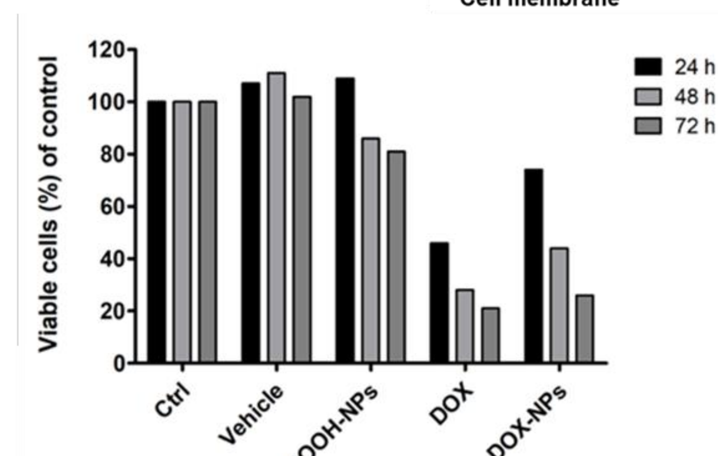


Results

The recognizing capacity of the antibody conjugated SiNP was investigated by means of flow cytometry. The cytogram below shows that the Ab-SiNP construct specifically binds to T cells as well as the conventional counterpart. In addition, the amplification effect of the SiNPs is confirmed by the increase in fluorescence intensity compared to antibody conjugated to a conventional tandem dye.
The differential behavior between free doxorubicin and SiNP conjugated doxorubicin was stated using fluorescence microscopy. The main difference observed is the preferential localization of SiNP conjugated doxorubicin outside of cellular nucleus which induces a lower cytotoxic effect. This characteristic behavior is probably due to the confinement of the construct in intracellular vesicles, as stated in our previous work.







Conclusions and Future Perspectives

The separated analysis of the two different components of the final construct allows us to determine important features for its future development. In particular, the exploitation of a cleavable linker for the conjugation of the drug to the construct will be mandatory in order to allow the release of the drug directly on the site of action (properly recognized thanks to the confirmed antibody capacity).

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