Functionalization of colloidal nanoparticles with a discrete number of ligands based on "bio-click reaction"

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The ability to develop nano-conjugates carrying bioactive ligands on the surface with an increasingly accurate control over the structure represents an important issue for nanotechnology. The design of an ideal nano-conjugate requires optimization of fundamental parameters including size, shape, shell composition of the nanoparticle and the possibility to stably conjugate a discrete number of ligands on the surface. In this perspective, among a huge number of diverse conjugation approaches, those belonging to the "bio-click reactions" group are believed to be the most promising techniques.

In this work we develop a general method to obtain stable gold nanoparticles functionalized with a discrete number of biologically active molecules, in particular one and two biomolecules for each particle, using the "bio-click" approach. This peculiar method relies on the use of bi-modular recombinant proteins composed of two portions: a first main module that provides the biological functionality desired for the nano-conjugate and a second enzymatic module that serves for the "capture" and the linkage with the nanoparticle. The enzymatic module is properly designed to bind a small molecule (its substrate) suitable to be immobilized on the surface of the nanoparticle, allowing the formation of a covalent, irreversible and oriented bond between the nanoparticle and the protein of interest.

For this project we chose to use a fusion protein made of the engineered enzyme Halo (*Haloalkane Dehalogenase*) as second module and the GFP (*Green Fluorescent Protein)* as first module, used for the characterization studies of the bioconjugate. The intrinsic fluorescence property of GFP in fact allows a direct quantification of the link between nanoparticle and biological ligand as well as offering indication on the maintenance of the biological functionality, which is a critical issue in the field of bio-functionalization.
Spherical gold nanoparticles with a diameter core of 4nm were used for this study; after the functionalization reaction, nanoparticles that bring a different number of molecules on their surface were separated using the electrophoresis technique. The nano-constructs were finally characterized by spectrofluorimetry.
The results obtained show that we have been able to develop a general model for the functionalization of colloidal nanoparticles with a controlled and discrete number of biological molecules through a conjugation approach that meet all the key requirements for an optimal bioconjugation. Indeed, since the "*Halo* module" can be produced in principle in fusion with any type of peptide or protein, this strategy may have general applicability resulting suitable for the development of ideal nano-constructs for active targeting in drug delivery.