

How the number of antibodies attached to colloidal nanoparticles affect tumour targeting and therapeutic effect?

Miriam Colombo

University of Milano Bicocca

Colloidal nanoparticles (NPs), including metal, magnetic and semiconductor NPs, are a versatile tool to integrate nanotechnology and biology, provided that they are complemented with a proper surface functionalization. Active targeting of nanoparticles to tumors can be achieved by conjugation with specific antibodies. While several studies have been carried out to achieve a control on linkage stability, as on ligand orientation and density, besides some examples a general strategy to introduce a discrete precisely controlled number of targeting biomolecules to each NP is still largely missing. Importantly, this makes it difficult to provide direct evidence on the relationship between the extent of NP functionalization and the targeting efficiency of the NP as a selective diagnostic tool or a drug delivery system.

We developed a nanostructured probe consisting of colloidal polymer-coated Au NPs functionalized on their surface with a defined discrete number of trastuzumab molecules. Specific active targeting of the HER2 receptor is demonstrated *in vitro* and *in vivo* with a subcutaneous MCF-7 breast cancer mouse model with trastuzumab-functionalized gold nanoparticles. The number of attached antibodies per nanoparticle was precisely controlled in a way that each nanoparticle was conjugated with either exactly one or exactly two antibodies. As expected, *in vitro* we found a moderate increase in targeting efficiency of NPs with two instead of just one antibody attached per nanoparticle. However, the *in vivo* data demonstrate that best effect is obtained for NPs with only exactly one antibody. There is indication that this is based on a size-related effect. These results highlight the importance of precisely controlling the ligand density on the nanoparticle surface for optimizing active targeting, and that less antibodies can exhibit more effect.