Featuring peptides for highly stable and stoichiometrically functionalized biohybrid nanoparticles for biomedical applications

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Nanotechnologies are leading an ever changing map of biomedical research and seek to provide solutions for the treatment of human diseases. Inorganic nanoparticles, with their physical, chemical and optical properties, have a large impact in biological applications. However, passivating nanoparticles and controlling their functionalization remains challenging and is a crucial requirement for successful *in vivo* applications. Here, we present the potential of well-designed peptides for the stabilization and passivation of inorganic nanoparticles, as well as stoichiometric functionalization with biomolecules. Finally, peptide shells were used for the preparation of novel biohybrid nanoparticles and biological probes.

I. Introduction

In this paper, we address a major gap separating inorganic nanoparticles from *in vitro* and *in vivo* applications. Biocompatibility, i.e., stability, specificity and functionality with the surrounding environment, is a crucial feature that defines the fate of any nanomaterial as a biomedical tool. This can be provided by the use of well-designed short peptides that form a protective layer on the surface of inorganic nanoparticles.

II. Peptide Design for Nanoparticles Passivation

Short peptides with a 'foot-stem-head' structure, have been synthesized to form self-assembled monolayers, efficiently stabilizing inorganic nanoparticles. Mixtures of thiolated peptidols, e.g., H-CVVVT-ol, and thiolated alkane polyethylene glycol ligands have been selected from large libraries of designed ligands to efficiently protect small noble metal nanoparticles. Their use prevents undesired electrolyte-induced aggregation and unspecific binding.[1], [2] These results show that it is possible to efficiently control the interface between inorganic nanoparticles and their environment with peptide ligands.

III. Functionalized Peptide Shells for Specific Probing and Biohybrid Nanoparticles Preparation

Recently, we showed that well designed peptide ligands are a promising platform for the preparation of targeted biological probes and biohybrid materials.

Stoichiometrically functionalized gold nanoparticle probes have been recently prepared with a previously described sphingolipid binding domain peptide (SBD). *In vitro* tests showed, by photothermal microscopy, specific targeting of lipid rafts (Fig. 1).

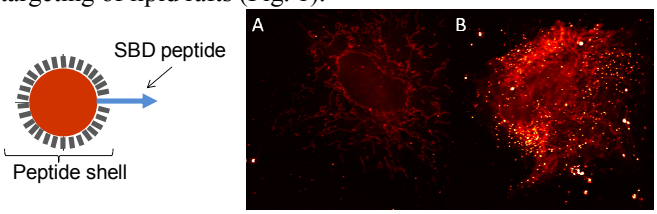

Figure 1. Monofunctional SBD-gold nanoparticles visualized *in vitro* by photothermal microscopy on R27 cells. A/ Non-functionalized gold nanoparticles show no non-specific binding. B/ SBD-gold nanoparticles probe membrane microdomains.

Multifunctionalized peptide ligand shells were successfully used to prepare stable biohybrid gold nanoparticles with the protein cage E2.[3] Mutants of the protein cage E2 have been prepared to encapsulate peptide coated gold nanoparticles exposing appropriate functions on their surface, providing a new route for the preparation of perfectly structured biohybrid nanoparticles (Fig. 2).

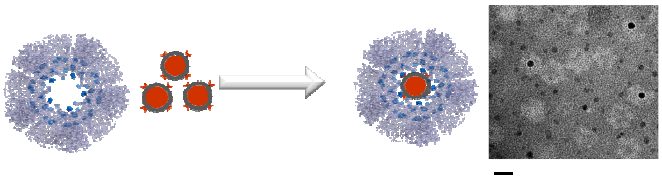

Figure 2. Biohybrid nanoparticles preparation with multifunctionalized peptide coated gold nanoparticles and protein cage E2 mutants. Scale bar of HR-TEM is 20 nm.

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References

[1] X. Chen, W. W. Qoutah, P. Free, J. Hobley, D. G. Fernig, and D. Paramelle, "Features of Thiolated Ligands Promoting Resistance to Ligand Exchange in Self-Assembled Monolayers on Gold Nanoparticles," *Australian Journal of Chemistry*, vol. 65, no. 3, p. 266, 2012.

[2] P. Free, D. Paramelle, M. Bosman, J. Hobley, and D. G. Fernig, "Synthesis of Silver Nanoparticles with Monovalently Functionalized Self-Assembled Monolayers," *Australian Journal of Chemistry*, vol. 65, no. 3, p. 275, 2012.

[3] D. Paramelle, N. Tomczak, P. Free, S. Lim, and T. Peng, "Specific internalization of nanoparticles into protein cages." Singapore Patent Application No. 201209135-1., 2012.