Heterogeneous Surface Coatings Engineering by Stealth-like Biomimicking

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When nanomaterials are introduced into a biological environment, they interact with endogenous biomolecules, particularly proteins, leading to the formation of the well-known protein corona, which can be divided into hard and soft corona depending on the strength of interaction.[1,2] This dynamic adsorption process alters nanomaterial identity, affecting its stability, biocompatibility, biokinetics, immune response, clearance, and overall biological behavior. Thus, for biomedical applications, it is of paramount importance to improve our understanding of the formation of the protein corona and to gain control over protein-nanomaterial interactions, enabling successful applications. The creation of stealth behavior, or antifouling, which means avoiding nonspecific interactions with proteins and other biomolecules, is the first step to later gain more control over the specific nanomaterial-protein interaction by the incorporation of selective groups targeting molecules. Therefore, the stealth behavior without nonspecific interactions, and the specific interaction required for targeting become the two sides of the same coin.

Different coating strategies have been proposed to prevent the formation of the protein corona on specific materials, including, among others, the use of zwitterionic polymers, zwitterionic ligands, cyclic polymers, or peptides. But it is the amphiphilic polymer polyethylene glycol (PEG) that is by far the most used antifouling coating. The homogenous distribution of non-charged polar residues and the high mobility of the PEG chains produce a steric repulsion that eludes protein interactions, generating stealth behavior. Their use requires, in general, relatively high molecular weights, contributing to a notable increase in the size of the nanoparticles, and generating, among other effects, nanoparticles of sizes too large to be removed by the renal clearance system. For low molecular weights, there are still non-negligible interactions with proteins in the form of a soft corona.

Despite the difficulties in obtaining unspecific protein corona formation in small nanoparticles, this task is routinely carried out by endogenous globular proteins that travel through the bloodstream without interacting with other molecules while maintaining specificity for certain tasks. The heterogeneous surface of the nanoscopic proteins, which includes negative and positive charges, as well as apolar domains, plays a key role in their stealth properties. In this context, a biomimicking strategy has been proposed based on the use of nanoparticle surfaces with the same balance of charge types and hydrophobic domains as endogenous proteins.[3] Despite this promising and simplistic approach, given the complexity of proteins, it is expected that other parameters, such as surface curvature, ligand heterogeneity, or ligand properties (length, mobility, etc.), also play a significant role in the antifouling capabilities of those nanoparticles.

In this work, we have explored the formation of the protein corona on gold nanoparticles (AuNPs) stabilized by thiolated functional PEGs (with positive, negative, and apolar end groups), considering two aspects: the molecular weight of the used PEGs, and the effect of nanoparticle curvature by varying the core diameter from sizes of similar sizes to endogenous proteins to infinity curvature (flat surfaces). AuNPs with uniform sizes and different diameters were obtained by fine-tuning the citrate reduction in a modified Turkevich method and seed-mediated growth approach, while surface coatings were produced by ligand-exchange, which allows having samples and controls with the same size and size distribution. For the formation of heterogeneous self-assembled monolayers of PEG-SH and the homoligand controls, three different end-functional groups were chosen: $-NH_2$, -COOH, and $-OCH_3$). The ligand ratios were chosen in a way that they maintain the same charge balance as the surface of blood proteins such as Human Serum Albumin (HSA) and Apoferritin (Apo). The nanoparticle-protein interaction with control proteins such as HSA was studied by different techniques, such as Dynamic Light Scattering (DLS), UV-Vis spectroscopy, and nanoparticle tracking analysis (NTA), among others. On the other hand, the antifouling properties of biomimicking flat surfaces were assessed using Quartz Crystal Microbalance (QCM).
As expected, in both configurations, the length of PEG plays an important role in the interaction with proteins, with a clear increase in nanoparticle size upon protein interactions, for molecular weights below 3 kg mol^{-1} confirming the limiting application of PEG when small coating sizes are required In addition, preliminary results show that the protein biomimicking configuration presents a decrease in protein interaction when compared with the homoligand nanoparticle independently of the charge type. Finally, the effect of size was not straightforward, with a lower effect than the type of selected ligand coating. These coating strategies open the door to easy functionalization of different nanomaterials, different than gold, with the help of other binding groups, catechols or silanes, among others, as well as flat surfaces with superior stealth behavior and relatively thin coating thickness. The protein biomimicking with short PPEGs enables a considerable reduction in size for sub-10 nm nanoparticles, which has important implications in biokinetics, and opens the door for biomedical applications where the clearance of the excess of nanomaterials through renal systems is achieved. Their possibility to be used on flat surfaces also allows the formation of coatings of biomedical

devices and implants, in which the interaction with proteins and other biomolecules needs to be prevented.

References

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Figures

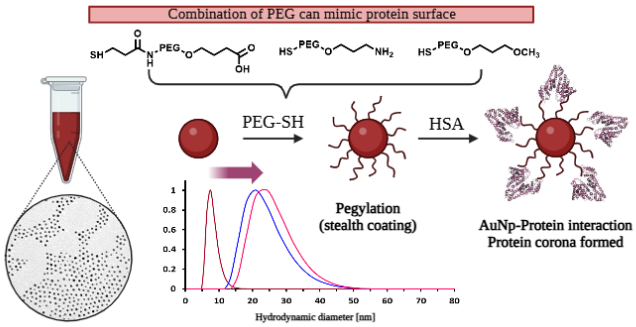

Figure 1. Schematic representation of gold nanoparticle functionalization through ligand exchange and measurements of nanoparticle-protein interaction through DLS measurements.