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Visualizing the Protein Corona: A Qualitative and Quantitative Approach towards the Nano-bio-interface

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The use of nanocarriers in biology and medicine is complicated by the current need to understand how nanoparticles interact in complex biological surroundings. When nanocarriers come into contact with serum, proteins immediately adsorb onto their surface, enfolding the nanocarriers and forming a protein corona which then defines their biological identity. Although the composition of the protein corona has recently been determined by proteomics, its morphology still remains unclear. In this study we show for the first time how a protein corona is adsorbed onto nanoparticles using transmission electron microscopy. We are able to demonstrate that the protein corona is not, as commonly supposed, a dense, layered shell coating the nanoparticle, but on the contrary an undefined, loose network of proteins. In addition, we are now able to visualize and discriminate between the *soft* and *hard* corona using centrifugation-based separation techniques together with proteomic characterization. The process of compositional change in the protein corona was analyzed after each of the multiple centrifugation steps, and the protein composition of the hard corona could be determined, depending on the surface chemistry of the respective nanoparticle. This, in turn, will allow us to define the individual physiological response of any nanoparticle system used in medical applications.

On account of their small size, nanocarriers have distinct properties that make them excellent candidates for biomedical and biotechnological applications. Although their use is growing rapidly, crucial questions still arise about the interaction of nanocarriers with biological systems. When nanocarriers come into contact with biological fluids they adsorb proteins due to their high surface free energy [1-2]. The proteins that are adsorbed on the surface of nanocarriers form the so-called 'protein corona'. The protein corona thus formed alters the size, aggregation state and properties of the nanoparticles and provides them with a biological identity, which differs from their synthetic identity [3-4]. The corona forms rapidly [5] and the composition changes only quantitatively [6]. It divides into the 'hard' and 'soft' corona, depending on the binding strength and exchange rate of the proteins. The hard corona is formed by the proteins with high binding affinities that are tightly bound and the soft corona by those proteins that are loosely bound and have high exchange rates. What the cell is finally able to recognize is the particle-protein complex [7]. This means that the individual proteins present in each case are responsible for regulating the cellular uptake [8-9] and the intracellular fate [10].

In this paper we focus on three different polystyrene nanoparticles (plain, carboxyl-functionalized and amino-functionalized). These particles can be easily synthesized in a wide range of sizes/surface functionalization and are ideal candidates for studying bio-nano interactions [11]. The protein corona is not only determined by surface functionalities introduced by co-monomers, but also by the surfactants used. We used the surfactant Lutensol AT-50 with a polyethylene glycol (PEG) tail of 50 ethylene oxide units. In our article we provide data on the exact composition of the protein corona that is formed after incubation in human serum and after repeated washing/centrifugation steps. In addition, we provide a quantitative approach to determine the absolute quantity of proteins adsorbed on nanoparticles using two

different methods. These studies offer us a better understanding of the biological identity of the nanoparticles and will therefore contribute to a safer and more effective application in nanomedicine.

The research presented here was carried out on a defined set of polystyrene nanoparticles (PS-NP), synthesized by free-radical mini-emulsion polymerization [12], stabilized with the surfactant Lutensol AT-50. In contrast to other studies where particles with different properties were used such as material, size or charge [13-15] we focused on a set of PS-NPs with similar size and varying surface modifications (PS-Lut, PS-Lut-COOH, PS-Lut-NH_2). Surface functionalities were introduced by co-polymerization of monomers containing carboxy- and amino-groups. The physico-chemical properties of nanoparticles such as charge, shape and size were characterized by ζ-potential measurements, transmission electron microscopy (TEM) and dynamic light scattering (DLS) in an aqueous solution and physiological buffer conditions (PBS).

Complementary analytical methods were applied to visualize the structure (TEM) of the protein corona and determine changes in size. The methods included multi-angle dynamic light scattering (DLS) as well as characterizing its composition by label-free, ultra-pressure liquid chromatography mass spectrometry (UPLC-MS).

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