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How Protein Adsorption Shapes the Biological Identity of NPs – Where do we Stand?

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The still growing use of nanoparticles (NPs) and nanomaterials in scientific and commercial applications leads to an increasing release and accumulation of NPs in the environment. An additional source of human exposure to NPs is the development of NP based formulations for drug-delivery and diagnostic applications which is a fast growing area of contemporary research. These, intended and unintended, scenarios of human exposure to NPs have sparked a substantial interest in understanding the interactions of NPs with biological systems.

A central aspect of the biological response to NPs is the adsorption of proteins and other biomolecules onto NP surfaces and the consequences arising from this interaction process. Wherever NPs come in contact with biological systems, physical and chemical interactions take place between surfaces of NPs and different biological components (Nel et al. [1] presented an excellent discussion of the basic physical interactions occurring at the nano-bio interface). It is now well established that, upon NP exposure to an organism, proteins from body fluids bind to NP surfaces [2], so living systems really encounter NPs enshrouded with biomolecules rather than bare particles. This so-called "protein corona" forming around the NPs largely defines the biological identity of the NP, and the efficiency of this interaction can be a decisive factor of the biological response of an organism to NP exposure [2-5].

The formation of the protein corona is essentially a competition of proteins and other biomolecules for binding to the NP surface. Whilst highly abundant proteins will likely dominate the protein corona at early times after exposure, proteins with a lower abundance but higher affinities might prevail on longer timescales.

The protein corona affects the colloidal behavior of the NP itself but can also affect the proteins' structure and function as well as the cellular uptake of the NPs. Understanding the formation, persistence and consequences of the protein corona is a complex task and of great importance for the elucidation, interpretation and assessment of the biological effects of NPs.

Role of NP Properties

The physical and chemical characteristics of NPs determine their interactions with the surrounding medium by promoting the adsorption of ions, proteins, natural organic materials and detergents, particle dissolution or even by allowing the free surface energy to be minimized by surface restructuring [1]. We now know that the surface chemistry of NPs is a decisive factor for the binding of proteins to their surface and the presence of polymer coatings around metallic NPs was shown to markedly affect the protein binding affinity to the NP surface [6,7]. Whilst typically binding affinities in a micromolar concentration regime were found for protein adsorption onto polymer coated NPs [3,6-9], binding onto freely accessible metal surfaces is much stronger with usually nanomolar binding affinities [6,7,10]. This emphasizes that the persistence of the surface functionality is an important parameter affecting protein corona formation in the physiological context. Only a stable molecular surface functionality on the NP surface under physiological conditions will be able to play a significant role in shaping the *in vivo* protein corona.

In addition to surface chemistry, NP size was shown to be an influential factor for the formation and composition of the protein corona in human serum [11]. The current absence of a reliable molecular scale interpretation for such results further underlines the complexity of protein corona formation and the dire need for mechanistic explanations.

Colloidal stability of NPs was shown to be strongly affected by the formation of a protein corona which was generally observed to have a stabilizing effect on the colloid by proving an additional steric stabilization [10,12]. However, the exact correlation between chemical and physical parameters of the NPs and the effect of the protein corona on their stability remains to be established.

A further issue that still requires attention is, if, and how different NPs are degraded in a biological environment. Degradation may not only remove the protein corona on the NP, it may also modify or remove the original surface functionality, and it may finally lead to an exposure of the NP core and even its complete dissolution. These processes can induce toxic effects by the release of molecular species or metal ions into the biological environment and the toxicity of degrading NPs will generally be a combination of ionic/molecular toxicity and toxicity aspects related to the particulate nature of the material [13].

An improved understanding of the biological effects of NPs requires a still more profound knowledge of the binding properties of proteins and other molecules that associate with the NPs. It is now recognized, how important a profound understanding of this protein corona is for shaping the surface properties, charges, resistance to aggregation and hydrodynamic size of NPs.

Impact on Protein

An important aspect of protein adsorption onto NP surfaces is that structural changes of the protein may occur, giving rise to altered protein conformations [14] that can also lead to a loss of protein function. Structural changes in the protein upon adsorption onto the NP surface may well lead to the exposure of novel "cryptic" peptide epitopes, altered function and/or avidity effects [15,16]. When a protein containing cryptic epitopes is denatured on a particle surface, the exposure of new antigenic sites may elicit an immune response, which, if launched against a self-protein, could promote autoimmune diseases [1].

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Whilst protein structure seems to be retained within the corona in many cases [3,8,9], a clear loss of protein structure has been demonstrated for other systems [2,6,10]. First mechanistic insights into the causes for this behavior were given, considering the involvement of specific functional groups [7] or Coulomb type interactions between the NP and charged patches on protein surfaces. However, many aspects of protein unfolding at NP surfaces remain still elusive [1].

Corona Influence on Cellular Uptake

NP uptake occurs mainly via the endocytic machinery of the cell [17]. In case of smaller NPs (~10 nm), it was shown that a critical threshold density on the cell membrane has to be exceeded to trigger the internalization process [18]. However, quantitative details of such threshold densities and their dependence on NP characteristics are still to be established.

Many studies reported effects of protein corona formation on the cellular response to NP exposure. For example, uptake of carboxyl-functionalized NPs by HeLa cells with adsorbed blood plasma proteins was shown to be strongly suppressed in comparison to bare NPs [8]. Immunoglobulin binding caused NP opsonization, thereby promoting receptor-mediated phagocytosis by macrophages [19]. Adsorbed proteins are internalized by the cells together with the NPs and may therefore enter cellular compartments which they would not normally reach [15]. Pathways for intracellular transport of NPs have also been revealed [20]. How these are affected by NP properties and by the presence of a protein corona around their surface is an important issue, needing close attention.

Moreover, NPs can penetrate cell membranes by processes that do not involve any active uptake machinery of the cell. The involved processes depend on the NPs' physical properties including size, surface composition, and surface charge [21-26]. While some NP types were shown to rupture the plasma membrane, eliciting noticeable cytotoxic effects [27,28], other studies demonstrated that NPs may enter cells without causing membrane leakage [29]. Again, little is known about the cause-effect relationship between NP properties and their behavior in passive uptake.

Conclusion

Conclusively, the current state of knowledge demonstrates an urgent need for further detailed studies of protein adsorption to NPs on the molecular level. Most importantly, the temporal evolution from a weakly bound (soft) corona to a more persistent (hard) corona under physiological conditions needs a mechanistic explanation.

First correlations between NP properties and their behavior towards proteins and cells were established, however, they now need a mechanistic explanation to gain predictive power. Current findings indicate the dependence of the protein corona composition in biological fluids on NP surface properties. However, some of the original surface properties may well be modified in the biological environment, which illustrates the importance of the persistence of NP surface functionalities under biological conditions.

While large gaps still exist in the understanding of the fundamental physicochemical aspects of corona formation, even larger gaps exist in applying this knowledge to a realistic biological situation. The consequences of the protein corona (and its properties) on the biological behavior of NPs are still elusive and poorly understood.

Recent years have seen substantial progress towards a better understanding of the interactions at the bio-nano interface, although many details remain to be explored. The importance of specific interactions between NPs and cell surface receptors has been shown, but the effects of physical and chemical properties of the NP surfaces deserve further attention. The key relevance of the protein corona in modulating cellular interactions has been recognized, but still very little is known about the dynamics of protein adsorption onto NPs in complex biological fluids, protein conformational changes associated with corona formation and the ensuing effects on cellular responses. Considering the wide variety of proteins, NPs and cell types much work remains to be done until a more complete picture can emerge.

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