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Review article

# Understanding the nanoparticle–protein corona complexes using computational and experimental methods

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ABSTRACT

Nanoparticles (NP) have capability to adsorb proteins from biological fluids and form protein layer, which is called protein corona. As the cell sees corona coated NPs, the protein corona can dictate biological response to NPs. The composition of protein corona is varied by physicochemical properties of NPs including size, shape, surface chemistry. Processing of protein adsorption is dynamic phenomena; to that end, a protein may desorb or leave a surface vacancy that is rapidly filled by another protein and cause changes in the corona composition mainly by the Vroman effect. In this review, we discuss the interaction between NP and proteins and the available techniques for identification of NP-bound proteins. Also we review current developed computational methods for understanding the NP–protein complex interactions.



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## 1. Introduction

Nanoparticles (NPs) are recognized as particles having their all dimensions between 1 and 100 nm having different properties compared to their bulk materials.

Recent improvements of modern biotechnologies have increased the application of proteins in bio-medicine and bio-catalysis. It is noteworthy that protein activity depends on its flexible and sensitive conformations. Several factors such as pH, temperature, surface interaction, and also contaminants, may compromise the conformational and even the chemical structure of proteins (Wang, 1999). Instable structure of protein hinders their application as industrial enzymes for bio-catalysis, which are usually employed at high temperature. Protein within

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**Table 1**
Forces contributing to the interactions between proteins and NMs.

| Forces | Strength | Amount | Net contribution | Range [nm] | Specificity | Main factors |
|---|---|---|---|---|---|---|
| vdw | Weak | A huge number | Large | 0–10 | No | Interface complementarity |
| H-bond | Moderate | Many | Small | < 0 | Partial | Hydrogen donor/acceptor |
| Coulombic | Moderate | Many | Moderate | 0–10 | No | Charge state, ion strength |
| Hydrophobic | Strong | Some | Large | 0–10 | Partial | Hydrophobic surface |
| Π-Π Stacking | Strong | A few | large | 0–5 | Yes | Aromatic ring orientation |
| Salt bridge | Strong | A few | Moderate | < 1 | Yes | Multiple recognition |

nanomaterials provides a solution to overcome those barriers met by bio-medical and bio-catalytic application of proteins (Bi, 2012). Research on the NPs is currently one of the most active branches of science with the numerous applications in various fields including health, engineering science and technologies. For nanomedicine applications, the surface of NPs is covered by biological proteins (protein corona) and, hence understanding the interaction of nanomaterials with biological systems is of critical importance regarding their safe and efficient applications.

Corona formation is a dynamic and competitive process, where the proteins compete to be absorbed on the NP surface to form a bio-nano interface (Laurent and Mahmoudi, 2011; Bank, 2010; Mahmoudi et al., 2011; Lynch et al., 2009).

Composition of the NP-biomolecule corona depends on many factors such as size, shape, composition, surface functional groups, and surface charges of the NP; the nature of the physiological environment, i.e., blood, interstitial fluid, cell cytoplasm, and also duration of the exposure. The protein corona alters the size and interfacial composition of a nanomaterial, giving it a new biological identity which is what is seen by cells (Rahman et al., 2013).

Concentration of over 3700 proteins in plasma and the kinetic rates (binding and reaction constants) of each protein determine the composition of protein corona at any given time (Muthusamy et al., 2005). Sheng-Tao Yang et al. focused on the biosafety and bioapplication aspects of the protein-NP interaction. They summarized some guidelines to design the protein–nanoparticle interactions for obtain higher biosafety and bioapplications of nanomaterials. Many interactions such as Van der wals, H-bonds, electrostatic, hydrophobic, and Π-Π stacking contribute to protein adsorption on NP. The characteristics of these interactions are summarized in Table 1 (Yang et al., 2013).

## 2. Structure of corona

The adsorption of proteins on the NP surface is governed by protein–nanoparticle (P-NP) binding affinities as well as protein–protein interactions. Protein corona is constructed of different protein layers. The first layer of corona is being irreversibly fixed while the outer layers are less stable. Proteins that adsorb with high affinity, consisting of tightly bound proteins form the 'hard' corona while, and proteins that adsorb with low affinity consisting of loosely bounds form the 'soft' corona.

The hard corona proteins interact directly with the nanomaterial surface, while the soft corona proteins interact with the hard corona via weak protein-protein interactions (Walkey and Chan, 2012) However, none of them completely mask the surface of NP or its functional groups. In a study on dextran-coated superparamagnetic iron oxide NPs (SPIONs), the incubation of SPIONs in plasma and formation of the protein corona did not significantly chang the circulation lifetime (Simberg et al., 2009).

The composition and structure of the protein corona are characterized by five parameters; (1) thickness and density; (2) identity and quantity; (3) arrangement and orientation; (4) conformation; and (5) affinity. The thickness of protein corona can be a factor of many parameters such as protein concentration, particle size, and surface properties of particle. Most plasma proteins form a corona with hydrodynamic diameter of about 3–15 nm; thus, the coronas on these NPs are too thick to be composed of only a single layer of adsorbed protein and are composed of multiple layers (Walkey and Chan, 2012).

Entropy-driven binding is one of the adsorption mechanisms of protein on the NPs surface, however, it cannot change the conformation of proteins corona (Lynch et al., 2009).

The hard corona is thought to be more important than the soft corona to determine the physiological response to a nanomaterials (Lynch et al., 2007). Adsorption of protein from plasma can prohibit the nanomaterials against aggregation. Citrate-stabilized gold Nps aggregate immediately in phosphate buffered saline (PBS), but are stable in plasma (Dobrovolskaia et al., 2009).

Protein concentration is a key parameter. Plasma with low concentrations can reach the nanomaterial surface and prevent aggregation (Wiogo et al., 2011). Monopoli et al. investigated the silica NPs form aggregates in PBS containing plasma at low concentrations, while well-dispersed in PBS containing plasma at high concentrations (Monopoli et al., 2011). Lundqvist et al. have studied the 'hard' corona formed around NPs of different materials, including copolymer and polystyrene NPs, of different sizes, and with different surface properties (Lundqvist et al., 2008).

On the other hand, the proteins having weak interaction with the hard corona, and loosely bonded to the NP, form the soft corona. For some particles with functional groups, it is possible that they have only weak corona (Flanagan et al., 2011).

In contrast to the hard corona, structure and composition of soft corona is basically unknown, mainly since soft corona cannot be directly isolated and, thus it is a challenging issue. Most of studies examining the structure and composition of the soft corona rely on a limited array of in situ techniques or apply complex experimental procedures. Cedervall et al. examined shifts in the elution times of plasma proteins exposed to polymeric nanoparticles using size exclusion chromatography(SEC). Their analysis implicated 6 serum proteins of varying molecular weight presumed to be a part of the soft corona. The authors did not identify these proteins explicitly, but deduced that they are not albumin or IgG (Walkey and Chan, 2012).

## 3. Protein conformation

During adsorption on the NPs, proteins may undergo structural rearrangements called "conformational changes". These changes are thermodynamically favorable if they allow hydrophobic or charged sequences within a protein to interact with hydrophobic or charged surface of NPs. Binding of proteins to planar surfaces often induces significant changes in secondary structures while, highly curved surface of NPs can help proteins to retain their original structure. However, study of a variety of NP surfaces and proteins indicates that the perturbation of protein structure is possible. Lysozyme adsorbed onto silica NPs or bovine serum albumin adsorbed on Au NPs surfaces show a rapid conformational change at both secondary and tertiary structure levels (Vertegel et al., 2004; Shang et al., 2007). Most studies have reported that loss of $\alpha$-helical content occurs as detected by circular dichroism spectroscopy with