Nanoparticle-protein interactions: from crucial plasma proteins to key enzymes

Elodie Sanfins, Julien Dairou, Fernando Rodrigues-Lima and Jean-Marie Dupret

Université Paris Diderot-Paris 7, Unité de Biologie Fonctionnelle et Adaptative, CNRS EAC 4413, 75013 Paris, France

Email: fernando.rodrigues-lima@univ-paris-diderot.fr ; jean-marie.dupret@univ-paris-diderot.fr

Abstract. Studying the effects of NPs on proteins may help understanding potential biological injuries such as changes in protein fibrillation, exposure of new antigenic epitopes, and loss of function such as enzymatic activity impairment. In this mini-review we present recent data which help understand the basis of NP-protein interactions and their subsequent potential effects on key mediators of biological functions such as enzymes.

1. Introduction
In the recent past nanomaterials emerged and took their place in lots of fields, such as electronics, optics, cosmetics, food... Nanomaterials were also used in medicine and diagnostics. This dramatic increase of industrial production and use of nanomaterials has lead to investigate their potential health effects.

Indeed, the nanotoxicology is a field well established. Studies of the effects of nanoparticles (NPs) from different industry branches on cells and pathways are emerging, and most of the biological effects of NPs seem due to their interactions with proteins. Here, we review some articles showing the interaction between NPs and proteins, and the effects of these nanomaterials upon biological actors such as plasma proteins and enzymes.

2. Nanoparticle-protein interactions, corona and plasma proteins
When entering the body NPs are rapidly in contact with biological fluids such as plasma. In these physiological environments, NPs selectively bind proteins to form a coat known as the ‘protein corona’. Since this corona is likely to determine the fate of the nanomaterials in vivo [1], understanding how and why proteins are adsorbed to NPs is of prime importance.

The affinity of a protein towards NPs is modulated by surface properties of NP. In a given medium the surface properties are determined by NPs characteristics such as chemical composition, surface functionalization, the shape and angle of curvature, the porosity and surface crystallinity, heterogeneity, roughness and hydrophilicity or hydrophobicity. An important determinant of the nature of proteins adsorbed onto the surface of NPs is chemical composition of the nanomaterials which further modifies the biological fate and interactions inside living organisms. The study of the plasma proteins bound to single walled carbon nanotubes (SWCNT) and nano-sized silica indicated different patterns of adsorption. Serum albumin was found to be the most abundant protein coated on SWCNT but not on silica NPs, suggesting the importance of NPs composition in the formation of the corona [2]. Moreover, it has been shown that TiO₂, SiO₂, ZnO NPs of similar surface charge, bind to
different plasma proteins. For example transferin was found to bind only on the ZnO NPs and neither on TiO$_2$ nor SiO$_2$. Other important plasma proteins such as immunoglobulins, lipoproteins, coagulation and complement proteins were also found to bind differentially to these NPs. Furthermore the size and shape of TiO$_2$ NPs seemed to be important for the determination of the proteins bound to the NPs suggesting that the agglomeration state largely affected the protein binding pattern [3].

The forces that seem to be involved in the protein-NPs interactions are Van der Waals forces (related to charges that can be measured by the zeta potential of the nanoparticles), London dispersion forces (hydrophobic interactions), hydrogen bond acidity and basicity, polarization and lone-pair electrons. Recently it has been shown that the NPs-protein interaction mechanism can be predicted with the help of a mathematical model that is based on the assumption of an equal contribution of all inter-moleculars interactions [4]. Nanodescriptors can be created using the adsorption coefficients of probe compounds. These nanodescriptors represent the contribution and relative strengths of each molecular interaction. As an example, this model helped to predict for example the adsorption of various small molecules onto carbon nanotubes [4]. In summary, the affinity of nanoparticles to biomolecules is determined by the contribution of multiple site of adsorption on the NPs surfaces (that are close to the amino-acids residues of the proteins), and the contribution of various forces that depend on the protein and the type of nanoparticles. Predicting this kind of interaction could be very helpful in nanomedicine to eliminate certain type of nanoparticles based on their relative nanodescriptors [4]. Another example of a mathematical model has been described by Dell’Orco et al. This model describes the kinetics formation of the protein corona around copolymer NPs. Three plasma proteins were used: albumin, fibrinogen and high density lipoprotein. This model tends to be used to predict protein corona formation on NPs with other biological fluids [5].

Furthermore, the nanoparticles bound to protein can undergo dynamic changes as the particles enter onto or into cells. The NP-protein complex can persist in body fluids and cells from microseconds to days. As the environment of the NPs change while moving to another compartment or fluid, the composition of the protein corona is likely to also vary. It is interesting to note that high affinity proteins can displace the binding of low affinity proteins. Göppert et al. studied the NP-plasma protein interaction using 2-D gels approaches and showed that the protein adsorption pattern on solid lipid NPs was dependent on contact time [6]. This study confirmed the Vroman effect [7] that explains the time evolution of the corona: proteins with high affinities but in low concentration in the plasma can displace highly concentrated proteins that have low affinity for the NPs. When using N-isopropylacrilamide/N-ter-butylacrylamide NPs as a model, human serum albumin (HAS) was rapidly adsorbed onto NPs but soon replaced by apolipoproteins such as AI AII AIV and AV. In addition, given the high number of protein on the plasma, the equilibrium of the corona formation will not be reached immediately. [8]

Using Isothermal calorimetry (ITC), it seems that the HSA-NPs association is an exothermic reaction. Interestingly the highest surface coverage of HSA is achieved for the more hydrophobic particles. This shows that the surface coverage is strongly dependent on the particle hydrophobicity. It has been shown also that surface curvature of N-isopropylacrilamide/N-ter-butylacrylamide interferes with HSA binding. Indeed the protein adsorbed on flat surfaces tends to accumulate in multi-layers and can formed two dimensional structures. However for high curvatures, proteins are far from each other and tend to form one-layer around the NPs. [9]

Studying the effects of NPs on proteins give a plausible explanation and help understanding potential biological injuries, such as changes in protein fibrillation, exposure of new antigenic epitopes, and loss of function such as enzymatic activity impairment [10].

As pointed above, the biological fate and biodistribution of NPs are strongly dependent on the proteins that NPs meet in the competitive world of body fluid proteins and in particular in plasma. Interestingly, the proteins coated on the NPs depend on the composition, the surface and the size of the NPs. Therefore, it is important to study, which specific proteins bound to a certain kind of NPs, and mathematical models could help to predict it. It is then the NPs and the proteins coated to them that we have to consider for biological effects and not the NPs alone. Maybe this “corona” could helping
classify the NPs and even engineering NPs which give better responses when used as nanomedical applications.

**Figure 1.** Effects of NPs on proteins, and the forces of interactions. (Adapted from [10])

3. **Nanoparticle-enzyme interactions**

Like serum albumin, lysozyme is often used as a model in protein structural studies. In addition, the activity of lysozyme can be easily monitored. Studies of the interaction NPs-lysozyme can demonstrate that either the structure or/and the activity changes. This fact gives a better understanding and leads us to measure the interaction of NPs with the biological actors: enzymes.

Lysozyme is a small monomeric globular protein, formed of 129 amino acids. This protein has the ability of disrupting bacterial functions, including their membrane structure. The protein has α helix and β sheet domains with 4 disulfide bound. It contains 6 tryptophan (trp) residues and three of them in its active site. Two of them are located in a hydrophobic environment and one is separated from the others.

Studies show an evident conformational change when lysozyme interacts with NPs. For example, the lysozyme adsorbed on silver colloids show a loss in conformation, and more precisely the Ag NPs seems to interact with a trp and phenylalanine residues, situated in a loop in close proximity. Moreover it seems that Ag NPs interact rapidly with lysozyme, and as the time goes by, the molecule stabilizes itself on the NP. It seems also that the active site of the lysozyme is not affected by the adsorption, because the area adsorbed on the NPs is the N-terminal end of the protein. [11] ZnO NPs have been reported to modify the secondary structure of lysozyme. Indeed, with circular dichroism the content of α helix and β sheet has been determined in presence of ZnO NPs. As a matter of fact, α helix content increases at the expense of random coil. It also seems that lysozyme keeps its catalytic activity when
adsorbed to the ZnO NPs. Moreover, the protein resist to 8 M urea denaturation in presence of these NPs. NPs can stabilize the structure of the protein, increasing the $\alpha$ helix content, in the case of ZnO and lysozyme, preventing the urea to denature it and foreseeing the oligomerization of the protein. [12]

On the other hand, the interaction of lysozyme with NPs has also been described for TiO$_2$ NPs. Lysozyme seems to form bridges between the NPs and enhance the formation of aggregates. A change in the conformation of lysozyme was observed in the presence of TiO$_2$. Indeed the content of $\alpha$ helix decreased while the content of $\beta$ sheet increased, resulting in a loss of activity. [13]

With a different kind of NPs, CdTe coated with thioglycolic acid, it has also been seen that the secondary structure of lysozyme was changed during the binding process.[14]

Other proteins with catalytic activities were also studied in the presence of NPs. While NPs are often the cause of toxicity or loss of secondary structure, it seems that promising issues can be found in the NP-enzymes interactions. It has been shown that Horsereadish peroxidase and egg white lysozyme retained a high fraction of their activity upon binding to SWNTs. Furthermore the SWNT-enzymes conjugates seem to be more stable than the non conjugated enzyme in guanidine hydrochloride and at elevated temperatures [15]. In addition a model enzyme, soybean peroxidase, adsorbed onto highly curved surface of C60 fullerenes had an half time 13-fold higher than the native enzyme. Furthermore, this phenomenon is not unique to fullerenes, but can also be extended to other nanoscale supports including silica and gold NPs [16].

This stabilization versus inhibition of enzymes by nanomaterials has been studied with chymotrypsin [17]. Functionalized gold NPs exhibited different effects on chymotrypsin. There was no inhibition from the cationic NPs while anionic NPs were effective inhibitors, because of the electrostatic complementarities between the NPs and the protein [18]. Moreover, chymotrypsin has been found inactivated by carbon nanotubes. Not only those functionalized multiwalled carbon nanotubes bind to the chymotrypsin active site but they also competitively inactivate this protein enzymatic function [19]. In another study, Shang et al. show that silica NPs induce the unfolding of ribonuclease A (Rnase A). Moreover, this team showed that the thermodynamic stability of the Rnase A decreased upon adsorption onto the silica NPs, and that the size and curvature of this kind of NPs play a role in the stability of adsorbed proteins.

Interestingly, NPs have been shown to target the central nervous system [20] and can inactivate enzymes which have crucial catalytic activities. Acetylcholinesterase is a hydrolase of the neurotransmitter, acetylcholine, in cholinergic synapses and is one of the most important enzymes in the nervous systems. Wang et al. demonstrated the inactivation/inhibition of both acetylcholinesterase and butyrylcholinesterase by NPs in a dose dependant manner. Moreover this inhibition is due to the adsorption of the enzyme on the NPs [21].

More recently, the effects of NPs on major xenobiotic metabolizing enzyme have gained interest in the scientific community since these enzymes catalyze reactions of pharmacological and toxicological importance. The effects of polystyrene and silver NPs on certain cytochrome P450, (phase I XME), have been studied. Fröhlich et al. show that there is a size dependent effect of polystyrene NPs on biotransformation activity of various cytochrome P450 enzymes. They have also shown the interference of NPs on the cleavage of different synthetic substrates in the presence or in absence of cytochrome P450 inhibitors. Smallest polystyrene NPs (20 nm) have the highest effect on these xenobiotic metabolizing enzymes, due to the hydrophobicity of this kind of NPs and the adsorption of the enzymes on them [22]. In another study it has been shown that silver NPs of 15 nm diameter show an inhibition of 95% of activities of human CYP1A2, CYP2C19 and CYP3A4 [23].

Current studies in our laboratory confirm that certain NPs may have an impact on the biotransformation of pollutants by others XMEs.

4. Conclusion
The NP-induced protein modifications are promising fields for future research. Proper understanding of such phenomenon is further emphasized by the fact that these materials are utilized for diagnostic
and therapeutic purposes. In this article we reviewed the scientific data on the deleterious effects of NPs on key mediators of biological functions such as enzymes. Only a few studies are conducted on the crucial metabolism enzymes and enzyme dysfunctions, which are related to various pathologies [23-27]. It is crucial to understand how NPs are able to bind proteins, and how this corona can help NP internalization and interaction of NPs with intracellular compounds.

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