CHAPTER 1

Nanoparticle–Protein Corona Complex: Composition, Kinetics, Physico–Chemical Characterization, and Impact on Biomedical Applications

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1.1 Introduction

Due to recent advancements in the area of materials science, it is possible to prepare nanomaterials with precise shapes, sizes, compositions and surface modifications with desired ligands. The use of such fascinating materials has offered significant advances in the development of nanomedicines such as therapeutics and diagnostics.[1] To achieve the biomedical applications possible, these nanomaterials (NMs) are required to be administered to the human/animal body, which leads to the exposure of these materials to the complex composition of biological fluids including a variety of salts, nutrients, and proteins. Interactions between the constituents of biological fluids

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and NMs may alter the physiochemical characteristics of the NMs. Therefore, it is essential to understand the interactions and predict the fate of NMs intended for the use of drug delivery and therapeutic applications for alteration in their systemic circulation, bio-distribution in different organs, and bio-availability at the targeted site. Although there could be several constituents affecting the properties of NMs, in this chapter we will only focus on the interactions of NMs with proteins dispersed in the complex biological fluids and their effects on the potential applications. It is well documented that dispersion of NMs in biological fluids facilitates the formation of a NM–protein complex, termed as a "protein corona". The protein corona (PC) consists of various proteins adsorbed on the surface of NMs. The composition of the PC is governed by the size, shape, surface charge, composition and surface capping molecules, therefore, potentially each nanoparticle with a unique set of PC constituents represents a new biological identity.

The PC established at a nanoparticle (NP)–protein boundary can be broadly divided into two parts: the hard corona and soft corona. These forms of PC can be differentiated by considering the binding strength and rate of exchange of proteins from the surface of NMs.[2] Proteins constituting a hard corona are reported to have a high binding affinity with the NM's surface and, therefore, their rate of exchange is extremely slow but faster than the time needed for the internalization of NPs.[2] Such an adsorption behaviour of protein has been ascribed as irreversible binding.[3] Contrary to this, the soft corona consists of proteins that are loosely attached to the NP surface or sometimes associated with an already-formed hard PC by a weak protein–protein interaction.[4,5] Proteins in a soft corona display a rapid exchange of molecules from the surface of the NP and are, therefore, substituted easily in a biological environment of dispersed proteins. It has been reported that in a typical PC, the hard corona proteins are arranged in the inner layer of the corona whereas the soft corona proteins distribute them in the upper layer of the corona.[6] In a soft corona, outer layer proteins exhibit a high exchange rate due to the abundance of proteins in a biological fluid as well as due to their direct contact.[7] The binding affinity between proteins and the NP surface dictates the stability of the PC, where the soft corona is considered less stable than the extremely stable hard corona.[6] Formation of a hard or soft PC depends on the size, surface charge, morphology, and association/dissociation rate of proteins from the NPs.[8,9]

Adsorption and desorption of a protein/biomolecule to the surface of NPs is a dynamic process.[9–12] The association and dissociation rates of proteins can be represented by the factors k_{on} and k_{off}. The balance between k_{on} and k_{off} provides some important clues about the affinity of a protein/biomolecule for a NP, which is commonly referred to as "dissociation constant" (K_d).[9–12] The value of k_{on} is dependent on the contact frequency of the protein/biomolecule with NPs.[10–13] It has been observed that high concentrations of protein/biomolecules will diffuse quickly and thus display high k_{on} values. However, the value of k_{off} could be influenced by the binding energy of the protein–NP complex. Proteins with higher binding energies show lower k_{off} values.

In complex situations, proteins compete for binding sites available on NP surfaces through adsorption, which depends on their distinctive individual K_d values.[9–12] In general, K_d values calculated for the adsorption of certain proteins in isolation to NPs are reported to be in the range of 10^{-4} and 10^{-9} M.[10,14] This interaction could correlate well with antibody–antigen interactions. Recently, Tenzer *et al.* reported that the composition of the corona is mainly altered quantitatively but not qualitatively over time.[15] Older models suggest that the highly dynamic PC composition changes over time owing to constant protein association and dissociation events and is controlled by the Vroman effect.[8,16,17] However, new models assume that pristine NPs can exist only for a short time in a complex environment; however, the developed PC is arranged in the form of multiple core–shell structures leading to a 'Christmas tree-like' structure.[18]

1.2 Physico–Chemical Parameters of NPs Controlling Protein Corona Formation

Although it is well known that as soon as any NP is dispersed in a protein-rich solution, it undergoes spontaneous protein corona formation; however, certain physico–chemical aspects of NPs control the composition of the PC.

1.2.1 Protein Corona Composition Varies with NP Type

Considering the affinity of certain materials towards the particular functional group present in proteins, the composition of PC can be different for different NMs. Using commercially available NMs such as ZnO, SiO_2, and TiO_2, Deng *et al.* studied the binding efficacy of proteins present in human blood plasma. Considering the same surface charge, they found that SiO_2 NPs adsorbed a distinct group of proteins (*e.g.*, alpha-2-acid glycoprotein, apolipoprotein D, clusterin), which are significantly different from those adsorbed by ZnO NPs (haptoglobin-alpha, Ig heavy chain alpha, transferrin).[14] It has also been suggested that the library of proteins making PCs is not only controlled by protein–NP interactions but also protein–protein interactions. An experiment was performed using SiO_2 NPs interacting with the top 10 proteins adsorbed on the SiO_2 surface as well as the most abundant protein in human blood plasma, albumin. The results revealed that apart from these 10 proteins, other proteins, such as complement proteins, fibrinogen, and immunoglobulins (Ig), are also found in the PC, which significantly affects the opsonisation, circulation, and targeting efficiency of NMs.[4,19]

1.2.2 Effect of NP Size Over Protein Corona Formation

It has been reported that the dimensions of NPs significantly control the PC composition.[20] Among the different dimensions, the surface curvature of NPs is considered as an essential parameter, which facilitates the adsorption

and change in conformation of proteins and thus the composition of PCs.[4,21] It has been reported that since the surface curvature of NMs is higher than their bulk counterparts, the binding affinities of protein molecules remain different for materials of a similar composition.[22] Further, protein–protein interactions are decreased at highly curved surfaces resulting in a diverse composition of PCs. It has been shown that NMs with high surface curvatures induce a lower alteration of corona composition than proteins binding onto the flat surfaces of materials with a similar composition.[23] Tenzer *et al.* studied PC formation on SiO_2 NPs of different sizes when dispersed in blood plasma.[15] They observed that the size of the SiO_2 NPs significantly affects the binding of ~37% of the identified proteins. It was found that a variation of 10 nm in particle size leads to a significant change in the composition of PCs. Similarly, clustsacaerin lipoproteins were found to have a better affinity towards smaller SiO_2 NPs whereas prothrombin or the actin regulatory protein (gelsolin) showed absorbance on larger SiO_2 NPs. Authors also report that the adsorption of some proteins such as IgG or actin was not associated with NP size. Dobrovolskaia *et al.* observed that gold NPs (AuNPs) of 30 and 50 nm incubated in blood plasma resulted in the adsorption of more proteins on the smaller sized particles than larger AuNPs.[24] Additionally, Piella *et al.* reported that the thickness and density of PCs is predominantly dependent on the size of NPs. The diverse temporal patterns for the development of protein coatings are generally faster for smaller particles than larger particles.[25]

1.2.3 Effect of NP Shape on Protein Corona Formation

NP shape is another important parameter, which controls composition and overall PC formation. In this concern, García-Álvarez *et al.* have reported that 70 nm gold nanostars (AuNSs) showed a high concentration of absorbance of proteins due to the greater surface area of AuNSs than that of gold nanorods (AuNRs).[26] The shape of AuNPs has an immense influence on their interactions with cell layers; in particular, spherical AuNPs have a better adsorption tendency with mammalian cells than rod-shaped AuNPs.[27] Deng *et al.* studied the influence of titanium dioxide NPs' shape on protein binding and found that clusterin and apolipoprotein D have a binding affinity for spherical NPs but not for nanorods or nanotubes.[14] Additionally, Ma *et al.* have revealed the adsorption kinetics of human serum albumin (HSA), γ-globulin, fibrinogen (HSF) proteins, and RBC hemolysis when exposed to two different spherical and rod-shaped mesoporous silica NPs (MSNPs). They observed that the initial adsorption rate for HSF was high for rod-shaped MSNPs.[28]

1.2.4 Effect of Hydrophobicity and Hydrophilicity on Protein Corona Formation

Hydrophobic interactions and hydrophilic repulsion are the two major phenomena of aqueous media and they, therefore, significantly influence the adsorption and desorption of proteins as well as attenuate these processes

over a NP surface. Roach *et al.* reported that NPs bearing hydrophobic surfaces demonstrate better protein adsorption than NPs with hydrophilic surfaces. They observed that hydrophobic surface NPs are frequently involved in the denaturation of surface-adsorbed proteins and also cause a conformational change in the native structures of proteins.[29] Similarly, Cedervall *et al.* and Gessener *et al.* have shown that during the development of the PC, hydrophobic NPs display a higher binding affinity for apolipoproteins; however, hydrophilic NPs favour the adsorption of fibrinogen, IgG and albumin.[12,30] Cedervall *et al.* have further demonstrated that there is a direct correlation between an increase in protein stoichiometry and an increase in hydrophobicity. The residence time of albumin on hydrophobic particles was found to be shorter than on hydrophilic particles.[7] Additionally, Dominguez-Medina *et al.* have demonstrated that citrate-capped AuNPs dispersed in aqueous solutions undergo aggregation followed by precipitation due to the interaction of NPs with oppositely charged ions in solutions of similar pH and ionic strength to human blood plasma.[31] Colloidal stability was improved by the association of BSA molecules on the surface of negatively charged (citrate coated) AuNPs. Hydrophobic interactions between BSA molecules present on the AuNP surface could impart colloidal stability after corona formation. Hulander *et al.* studied the capability of pre-adsorbed IgG on AuNPs to elicit the immune complement (IC) after modification in surface hydrophobicity.[32] They found that surface-bound hydrophilic AuNPs diminished the ability of IgG to activate the IC. In contrast, an increase in surface hydrophobicity favours the activation of IC by IgG.

1.2.5 Effect of a NP's Surface Charge on Protein Corona Formation

The charge present on the surface of a NP is an essential feature for the determination of PC composition and formation. The interaction between the charges of the NP and protein molecules determines the adsorption kinetics and composition of a PC. For example, opsonins can promptly identify the NPs with a positive surface charge, which leads to recognition of the NPs by the reticuloendothelial system (RES) and subsequent deposition in organs such as the liver and spleen.[23,33] Considering this, it has been suggested that opsonization can be avoided by coating the NP's surface with negatively charged groups, which exhibits a negative zeta potential varying from −30 to −50 mV in a biological environment. Ehrenberg *et al.* have shown that NPs dispersed in a physiological environment lead to the adsorption of proteins, which can induce a remarkable drop in surface charge. This observation suggests that there is a direct correlation between the nature of the PC and the colloidal stability of the developed NP–protein complex.[34] In a further study, Lynch *et al.* have reported that AuNPs bearing positive, negative and neutral charges could induce protein denaturation to a different extent. Charged AuNPs (positive or negative) were found to cause a higher protein denaturation than a neutral charge thus

allowing the proteins to retain their native structure.[35] Similarly, Gessner *et al.* studied the surface charge density effect of polymeric NPs bearing a negative charge and observed that as the surface charge density increases, the plasma protein adsorption also increases.[36] Moreover, it has been revealed that for polystyrene NPs, proteins with an isoelectric point of <5.5 are likely to bind on the positively charged surface, whereas proteins with an isoelectric point >5.5 prefer negatively charged NPs.

1.2.6 Effect of Temperature on Protein Corona Formation Over NPs

Although the average internal temperature of the human body is considered as 37.0 °C (98.6 °F), it differs from person to person, based on various factors such as body type, gender, and physical activities. Additionally, a person cannot have a constant body temperature throughout the day. However, following a person's circadian rhythm, the cycle of the temperature of the body varies throughout the day and night. It is reported that exposure of parts of the body to cold surroundings could lead to a drop in body temperature to 28 °C.[37] It has also been reported that the temperature of the intracellular parts of living cells is not similar. The temperature of the female body is little higher compared to the male body, which varies further according to the female hormone cycle.[38] Physical activity can also lead to an enhanced body temperature by up to ~2 °C and the body temperature decreases during sleep. In general, the body temperature varies from 35 to 39 °C; however, during a fever, the body temperature can increase to 41 °C.[39] Therefore, it is also expected that PC formation may also be greatly influenced by varying the temperature of the surrounding environment. In this context, Mahmoudi *et al.* have studied the influence of alteration in temperature on the PCs formation and its concomitant composition.[40] Here, dextran-coated FeOx NPs with different surface charges were incubated with FBS at various temperatures and the authors found that it was not only the composition of the PC that was significantly affected but also that the pattern of cellular uptake was altered. Mahmoudi *et al.* reported that the composition of the PC on AuNRs can be influenced by the plasmonic heat induction.[41] Here, cetyltrimethylammonium bromide (CTAB)-coated AuNRs were incubated with different FBS concentrations and a change in PC formation was monitored prior to and after plasmonic heating by constant exposure to a laser. The alteration of the PCs composition on AuNRs was found to be dependent upon the type of heating applied such as plasmonic heating or conventional heating.

1.2.7 Effect of pH on Protein Corona Formation

Altering the pH can also be regarded as one of the key factors in assessing the formation of a NP–protein complex. Variation in environmental pH may vary protein binding affinity,[42] which can induce significant modification of

the adsorbed protein pattern. During cellular uptake, NPs are exposed to biological fluids with varying pH, such as blood (pH 7.4), dispersion media (pH 6.9–7.4), intracellular fluid (pH 6.8), and lysosomes (pH 4.5–5.0). Once NPs are administered, they tend to enter the blood circulation and are eventually deposited in different organs.[43] It is well known that cancer cells/tissues generate an acidic microenvironment, thus represent specific types of groups of proteins that can alter the PCs around NPs. These events could lead to the altered property of NPs, which is necessary for the therapeutic application and biological availability of NPs. Understanding the effect of pH on the formation of a NP-PC complex decides the fate of NPs in a biological environment present in the body.[43] When isoelectric points and pH values are very close to each other, the NPs' stability is decreased due to the significant effect of pH on PC formation and its correlation with the surface charges of NPs. Considering the anionic nature of natural organic matter (NOM) and prokaryotic or eukaryotic cells,[44] it was observed that their interaction with positively charged NPs is predominantly electrostatic in nature. Conversely, Chen *et al.* have reported that the binding of bacterial exopolysaccharides on a AgNP surface or humic acid on TiO_2 is significantly reduced when the pH exceeds the IEP.[45] Such examples indicate that the electrostatic interaction between NPs and other biomolecules during PC formation is not the sole mechanism; rather, ligand exchange or hydrophobic interactions are also essential for NPs, for, *e.g.*, fullerenes adsorbing NOMs by hydrophobic interactions.[46,47]

1.2.8 Effect of Surface Modification of NPs and Protein Corona Formation

Strategies are required to prevent undesirable proteins being absorbed on NP surfaces that results in PC formation. Such events may negatively affect the targeting moiety of NMs that are surface modified for selective binding to tissues or organs. A method to modify the NP surface by incorporation with certain functional groups prevents NPs being caught by immune cells. Coating NP surfaces with polymers, such as polyethylene glycol (PEG), reduces the binding of proteins from biological fluids and also prevents recognition by the RES. By preventing the formation of the PC, significantly quicker transport of NPs across the endothelium can be obtained. Further, PEGylation with a controlled density of PEG on the NMs' surface makes them suitable for longer circulation in the bloodstream. Gref *et al.*, Perry *et al.* and Engin *et al.* showed that silicon can be used as a coating material exhibiting a similar-favourable impact on PC formation.[48–50] Polystyrene coated with poloxam ine 908 indicated a drop in fibronectin adsorption compared to uncoated nanospheres.[51] Ehrenberg *et al.* demonstrated the interaction of polystyrene NPs with various functional groups on the endothelium cells. They found that the capability of the NPs' surface to adsorb protein is an indicator of their tendency to interact with cells and the bound proteins do not affect

cell–NP association.[34] Aggarwal *et al.* have shown that NPs coated with different molecules, such as PEG, poloxamer, poloxamine, dextran, pluronic F127, polysorbate and poly(oxyethylene) *etc.*, exhibited improved NP–protein interactions, protein conjugation and NP distribution in a biological system.[52] Dutta *et al.* reported that amorphous SiO_2 NPs and single-walled carbon nanotubes coated with pluronic F127 result in a better dispersion of NPs and also a considerable decrease in the adsorption of serum proteins.[53] Contrary to the aforementioned parameters, Muller *et al.* have reported the interactions of polymeric NPs and other inorganic NPs with plasma proteins obtained from different sources including human, rabbit, sheep and mouse.[54] The data obtained from liquid chromatography–mass spectroscopy (LC-MS) (Figure 1.1) shows that the composition of the PC differed when the NPs were dispersed in plasma proteins from the different sources.[54] Thus, it can be concluded that it is imperative to investigate the fate of NPs dispersed under *in vitro* and *in vivo* experimental systems by monitoring their interactions with plasma proteins from the corresponding cell culture or animal model. Additionally, reports suggest that the nature of the protein ultimately decides the fate of NPs in a particular organism.

1.3 Composition of the Protein Corona

Human blood plasma is the most abundant biological reservoir for PC formation. A layer of protein that covers a NPs surface influences its cellular uptake as well as biodistribution. In this context, Monopoli *et al.* have shown that fibrinogen, IgG, and complement factors lead to stimulation of phagocytosis and eventually elimination of NPs from the bloodstream; however, HSA and apolipoproteins extend the NPs circulation time in the blood.[55] Plasma proteins, such as fibrinogen, IgG, albumin, and apolipoproteins have a tendency for rapid adsorption onto metal-based NPs during the early phase of PC formation.[4,56] These proteins are observed in the hard coronas of all the studied NPs and are substituted by apolipoproteins and coagulation factors during the slow phase of corona formation. Walkey *et al.* introduced the term "adsorbome" to represent the group of 125 of the most prevailing proteins present in plasma.[17] Adsorbome is involved in a variety of physiological processes including lipid and ion transportation, activation of the complement system, recognition of pathogens, and blood clotting. Metallic NPs favour the adsorption of albumin, the most abundant plasma protein, during PC formation. At pH 7.4, albumin has a net negative charge due to ~60 positively charged amino acid lysine molecules, which facilitates the interaction of albumin with NPs (positive and negatively charged NPs). Irrespective of the net charge on the NP surface, albumins generally produce anionic PC complexes with NPs.[57] Additionally, Fleischer *et al.* have reported that the configuration of albumin protein could be altered by cationic NPs; however, anionic NPs did not show this effect.[57] An alteration in protein structure affects the interactions of the albumin–NP complex at a cellular level and, therefore, cell receptors bind to the PC on anionic NPs; however, albumin–NP complexes formed from

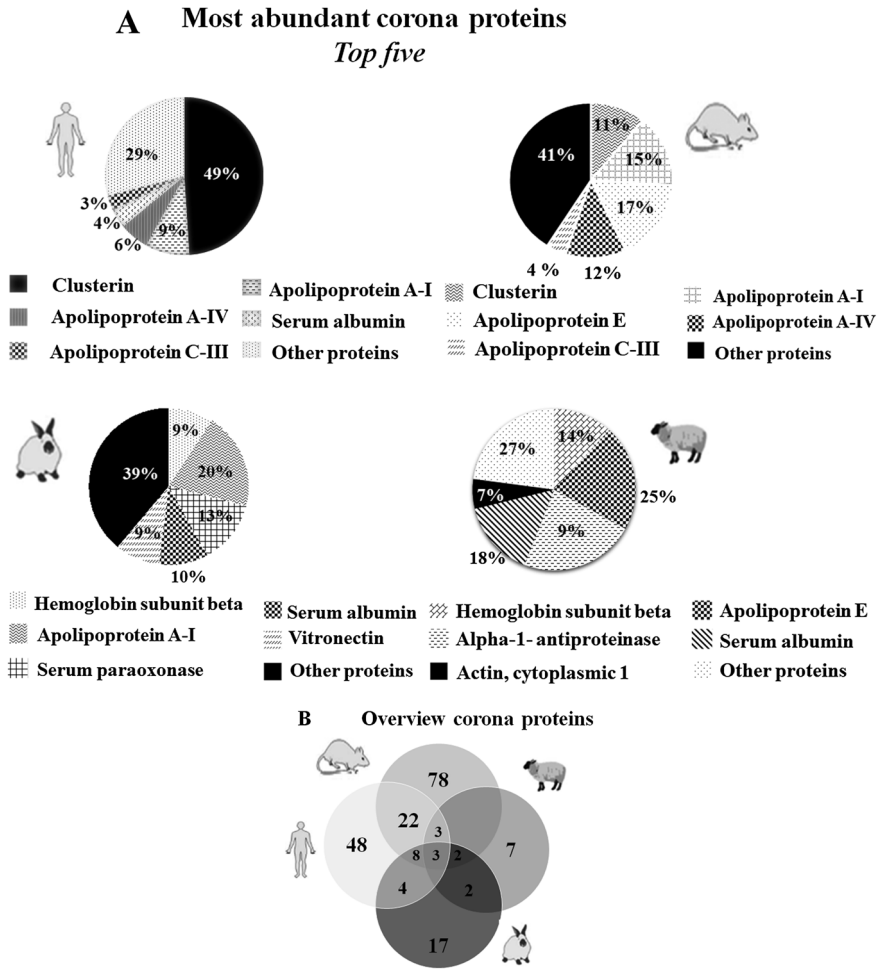


Figure 1.1 (A) Composition of the protein corona from different animal sources as determined by LC-MS exemplarily shown for un-functionalized PS-NPs. The five most abundant corona proteins based on all identified proteins are summarized. (B) Venn diagram showing common proteins from the list of all identified proteins per animal source for PS and PS-NH_2. Not shown are the number of common proteins from human and sheep (0) and mouse and rabbit (6). Reproduced from ref. 54 with permission from American Chemical Society, Copyright 2018.

cationic NPs are redirected to scavenger receptors. The adsorption of albumin was much better on the surface of anionic NPs compared to cationic NPs, leading to ~23% and 8% surface coverage, respectively. Albumin and fibrinogen are some of the most abundant proteins that constitute the PC composition on a variety of NPs. However, some proteins, such as apolipoproteins, show specific binding with other nanostructures, exhibiting a better adsorption affinity for liposomes and polymeric NPs, but a lesser affinity for metallic

NPs. A key factor required for the binding of proteins to NPs is hydrophobicity.[30] Therefore, polymeric and hydrophobic NPs facilitate the adsorption of proteins such as transferrin, haptoglobin, fetuin A, kininogen, histidine-rich glycoprotein, and intrinsic clotting pathway factors; however, most of these proteins can also show affinity for metal-based NPs[30] (Table 1.1).

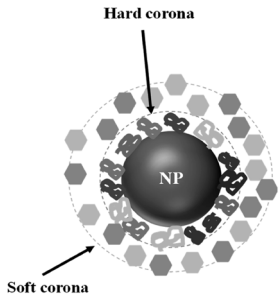
Sempf *et al.* have studied hard corona formation on the surface of uncoated poly-lactic-*co*-glycolic acid (PLGA) NPs incubated in human plasma and observed that 15 proteins were localized in the hard corona, consisting of seven that are not found in abundance in plasma. Apolipoprotein E, vitronectin, histidine-rich glycoprotein and kininogen-1 were the proteins from plasma adsorbed on the PLGA NPs and thus provided the best signal.[61] Further, Darabi Sahneh *et al.* also developed an experimental model explaining the two phase dynamics of a PC complex. They suggested the use of two formulae that provide information about the metastable and stable states of a PC. Mathematically, they could predict that the PC is independent of simulation by adding suitable values of parameters as well as understand the NPs' interaction with a physiological environment, applicable to toxicology and nano-based drug delivery applications.[62]

1.4 Kinetics of Protein Corona Formation

During the beginning of PC formation, the most abundant proteins but those with less affinity for the NP surface are the first to be adsorbed and develop a layer that is recognized as the soft corona. Subsequently, high affinity proteins tend to replace the low affinity proteins from the medium and thus develop a hard corona layer[7,12,33,55] In the case of the hard corona, a direct interaction of proteins occurs with the NP surface; however, in the soft corona, proteins interact with the hard corona proteins through weak protein–protein interactions. The kinetics of the formation of the soft and hard PCs differs significantly. The development of the hard corona is very rapid and occurs within seconds or minutes, whereas a longer duration (hours or even days) is required for the development of the soft corona, as higher affinity proteins are being substituted by lower affinity proteins. This procedure relies on the concentration of protein as well as the biological medium constitution.[17] Several evidences indicate that even if plasma concentration is low, corona proteins can totally mask the NP surface and thus alter its behaviour and physico–chemical characteristics.[55] Interaction of soft corona proteins with hard corona proteins is possible as they desorb from the NP surface, which provides an opportunity for the interaction of other biomolecules from the medium. The aforementioned exchange is attributed to the competitive adsorption and desorption of proteins, which can be influenced by several parameters such as interaction period, protein concentration, and adsorption efficiency of proteins with the NP. This exchange could be explained by the "Vroman effect"[52,63] and is comprised of two phases.

Table 1.1 A list of common plasma proteins found in PCs developed on different types of NPs.

Type of NP	Proteins identified in corona	References
AuNPs	Albumin, fibrinogen chains, apolipoprotein A1, transport proteins, coagulation factors	52, 58 and 59
SiO_2 NPs	Ig, lipoproteins, complement proteins, coagulation proteins, acute phase proteins, cell proteins, serum proteins	52, 55 and 59
Magnetic NPs	Albumin, apolipoprotein A1, complement factors, vitronectin, hemoglobin	59
Polystyrene NPs	Coagulation factors, Ig, lipoproteins, acute phase proteins, complement proteins, plasminogen, anti-CD4, C4a, albumin	52, 58 and 59
Latex NPs	Albumin, apolipoproteins, Ig, hemoglobin, haptoglobins	52 and 59
Copolymer NPs	Albumin, apolipoproteins, fibrinogen, Ig, C4BP-α-chain	59
Supraparamagnetic iron oxide NPs	Albumin, α-1-antitrypsin, fibrinogen chains, immunoglobulin chains, transferrin, transthyretin	56, 58 and 59
Carbon nanotubes	Fibrinogen chains, immunoglobulin light chains, fibrin, albumin, ApoA1, component proteins, fibronectin	52 and 58
TiO_2 NPs, ZnO NPs, SiO_2 NPs	Complement proteins, immunoglobulin light chains, fibrin, albumin, fibronectin albumin, Ig, fibrinogen, transferrin, apolipoprotein A1	52 and 59
Citrate-coated AgNPs	Albumin, α-1-antiproteinase, α-2-HS-glycoprotein, apolipoprotein A1, serotransferrin, α-macroglobulin, α-fetoprotein, apolipoprotein B100, α-2-antiplasmin, complement C3, β-2-glycoprotein 1, fetuin-B, inter-α-trypsin inhibitor heavy chain H1, hemoglobin foetal subunit β, inter-α-trypsin inhibitor heavy chain H3, inter-α-trypsin inhibitor heavy chain H2, hemoglobin subunit α, complement factor B, hemopexin, serpin A3-6	60
AgNPs coated with polyvinylpyrrolidone	Albumin, α-2-HS-glycoprotein, α-1-antiproteinase, apolipoprotein A1, serotransferrin, α-2-macroglobulin, α-fetoprotein, apolipoprotein B100, complement C3, α-2-antiplasmin, inter-α-trypsin inhibitor heavy chain H1, fetuin-B, β-2-glycoprotein 1, hemoglobin foetal subunit beta, inter-α-trypsin inhibitor heavy chain H3, inter-α-trypsin inhibitor heavy chain H2, vitamin D-binding protein, transthyretin, hemoglobin subunit α, complement factor B	60



Property	Hard Corona	Soft Corona
Timescale	Hours	Seconds to minutes
Protein affinity	Low	High
Protein binding strength	Protein weekly bound	Protein strongly bound
Interaction point	Protein to protein	Proteins to NP
Type of interaction	Protein to protein	Van der waals
Dissociation constant	High	Low
Conformation changes	Low	High
Proteins	Apolipoproteins	Albumin, apolipoproteins, IgG

Figure 1.2 Schematic representation showing the formation and properties of the two types of protein coronas: hard (inner layer) corona and soft (outer layer) corona.

During the first phase, proteins with the greater association rates adsorb quickly while during the second phase, proteins with short residence times are replaced by proteins with slower association rates but longer residence times.[64] Figure 1.2 represents the typical characteristics of a PC formed over the surface of a NP. Jansch *et al.* investigated the Vroman effect on ultrasmall superparamagnetic iron oxide (USPIO) NPs and revealed that the Vroman effect on USPIO cannot be measured and the exchange of high affinity proteins with abundant proteins on USPIO was also not detected. Furthermore, as the incubation time increased, the amount of adsorbed fibrinogen and Ig over the NP surface was enhanced while the relative quantity of key proteins, such as apolipoproteins, fibrinogen, and albumin, remained constant.[65] Winzen *et al.* studied a complementary investigation of the hard and soft corona using human plasma and individual proteins considering HSA and apolipoprotein A-I (ApoA-I). They observed that there was no significant alteration in hard corona formation. Though, the soft corona acquired some extra proteins, which were due to the differential binding affinity and other physiological factors of proteins.[2]

1.5 Common Techniques to Characterize Protein Coronas on NPs

Characterization of PCs is essential for understanding the mechanism of formation, kinetics, and their functional aspects. The common techniques required for the detailed characterization of PCs include UV-visible spectroscopy, Fourier-transform infrared spectroscopy, dynamic light scattering, zeta potential, circular dichroism (CD) spectroscopy, and isothermal titration calorimetry (ITC). These techniques are required to confirm the layer of protein present on the NP surface and are considered as primary characterizations. Further, the complete characterization of the PC can be performed by using advanced techniques such as electrophoresis, chromatography, mass spectrometry, and nuclear magnetic resonance, *etc.* In

this chapter, we will only discuss the primary characterization techniques for PCs; more detailed characterizations can be found in Mahmoudi *et al.*'s work.[23]

1.5.1 UV-visible Spectroscopy

Proteins absorb at a wavelength of 280 nm from UV-visible spectroscopy light.[66] Absorption of the UV light by proteins generally occurs due to the presence of aromatic amino acids, *i.e.*, phenylalanine, tyrosine and tryptophan. Binding of NPs to proteins changes the absorption spectra of NPs as well as the proteins, which is due to the electron transfer capabilities of the proteins with their optical properties.[13,67–70] Yadav *et al.*[68] utilized UV-visible spectroscopy to reveal the adsorption isotherm of lysozyme and BSA on anionic silica NPs and reported that adsorption of lysozyme can be correlated with the increase in the concentration. However, adsorption of BSA did not follow any adsorption pattern. In another study, it was observed that attachment of azurin protein on the surface of AuNPs makes a red shift in the surface plasmon resonance (SPR) of NPs, which was attributed to the energy transfer from azurin to the NPs' surface. Casals *et al.* reported that the SPR band of 10 nm AuNPs showed a red shift from 518 nm to 526 nm when incubated with a cell culture medium.[8] They reported that using UV-visible adsorption spectra, one can monitor the NPs' protein interaction by following the absorbance pattern of SPR. However, characterization by UV-visible spectroscopy alone is not enough because it is not very sensitive and, therefore, further characterization of protein-NP complexes is needed. Recently, we reported the formation of a PC on NPs and the observed changes were followed by different characterization methods including UV-visible spectrophotometry and transmission electron microscopy (TEM). In one of our studies, the typical absorbance of CeNPs (4+) was followed after dispersing them in MilliQ water and a DMEM cell culture medium (supplemented with 10% FBS). The results showed that CeNPs (4+) incubated in DMEM indicated two absorbance peaks at 280 and ~292 nm, which were ascribed to the presence of protein (from the cell culture medium) and CeNPs (4+), respectively.[71] Additionally, Vidic *et al.* have also reported PC formation over ZnMgO nanopowder when dispersed in a bacterial culture medium, luria bertani (LB), as well as the mammalian cell culture medium RPMI. The UV-visible absorption spectrum of ZnMgO dispersed in the LB medium displayed no optical features typical of ZnMgO nanopowder. The optical absorption recorded from the ZnMgO-LB suspension originates from components present in the LB medium such as amino acids, NaCl, and yeast extract. An intensity loss was observed in the spectrum after 24 hours, which indicates that the components were not present in free form; rather, they were attached to NPs. Similar to LB, NPs dispersed in the RPMI medium did not show any optical transitions characteristic of ZnMgO. After 24 hours of incubation of the ZnMgO NPs in complete RPMI medium, the absorbance intensity was reduced in the range of 200 and 300 nm except in the range of higher wavelengths. Thus, it can be concluded that

this intensity enhancement is probably due to the existence of new electronic transitions originated after the formation of complexes between ZnMgO and components of the medium.[72]

1.5.2 Fourier-transform Infrared Spectroscopy

Fourier-transform infrared spectroscopy (FTIR) is a technique that basically works on the vibration energy of chemical bonds, which can be used to get information on proteins adsorbed on the surface of NPs. Kong *et al.* explained the use of FTIR for the identification of a protein's secondary structure.[73] They provided a detailed discussion on the prediction of protein structure on the basis of the vibrational energy of peptide bonds and suggested that a protein's secondary structure can be studied on the basis of infrared light absorption by amide bond I (1700–1600 cm^{-1}),[23] II (1540 cm^{-1})[74] and III. Out of all the amide bonds, vibrations in amide bond I have been reported to be the most crucial.[73] FTIR spectroscopy has been used for the evaluation of protein coatings on the surfaces of NPs and the difference between the vibrational energy of protein-coated NPs in comparison to uncoated the NPs has been reported by various researchers.[67,69,70,75] Ma *et al.* used FTIR to study the adsorption of HSA, globulin (HGG), and fibrinogen (HSF) over MSNPs. The pore size and morphology of MSNs influenced the adsorption of HAS and HSF while adsorption did not alter the conformation of HGG.[28] Additionally, Shang *et al.* have also demonstrated the changes observed from the conjugation of BSA with AuNPs at varying pH (3.8, 7, and 9) using the FTIR technique. They observed that bio-conjugation of BSA with AuNPs leads to a conformation change probably due to the considerable enhancement in sheet and turn structures in BSA and thus a decrease in the helical structure component.[76]

1.5.3 Dynamic Light Scattering and Zeta Potential

Dynamic light scattering (DLS) or photon correlation spectroscopy is used to study the dynamic behaviour of fluids on the basis of their Brownian motion.[77] Plenty of research has been done on the characterization of protein-NPs using DLS.[24,67,78,79] DLS can be used to study the hydrodynamic size distribution of NPs that can be calculated from the time depending on the scattering intensity. Similarly, Casals *et al.*[8] used DLS to measure the hard coronas of proteins on the surface of AuNPs. The zeta potential of any solution shows its stability on the basis of electrostatic repulsion between similarly charged particles and it varies due to variation in dispersions.[80] The zeta potential values offer information about the stability of the particle. An increase in zeta potential values is associated with greater electrostatic repulsion and, hence, enhanced colloidal stability. It is important to consider that the magnitude of a NP's surface charge relies on the solution pH. In this context, Patil *et al.* reported that a change in pH from acidic to alkaline induces a significant change in the zeta potential value of cerium oxide NPs.[80] It was found that

with the incubation of cerium oxide with BSA, positively charged NPs exhibit a greater affinity for BSA adsorption compared to negatively charged NPs. It was also found that the cellular uptake of NPs varies with the surface charge of NMs. Zeta potential can be used to quantitatively measure the coating of proteins on the NPs' surface as with the growing protein coating, there will be a concomitant change in zeta potential.[8] Natte *et al.* investigated the adsorption of BSA on pristine silica NPs (SNPs) and silica polyethyleneglycol core–shell nanohybrids (SNPs@PEG) by measuring zeta potential.[81] Bare SNPs displayed a zeta potential of −21 mV, which remained the same when a lower BSA concentration was used to disperse the NPs. In contrast, a concentrated mixture of proteins lowered down the zeta potential up to −8 mV, similar to the zeta potential values of BSA dispersed in PBS. They found that the amount of BSA adsorption was dependent on the BSA concentration used as well as the incubation time with the NPs. Coating of PEG on the SNPs' surface was found to inhibit the short- and long-term formation of BSA PCs on SNPs, which was evident by the slight decrease in zeta potential due to the large polymer chain length of PEG.

1.5.4 Isothermal Titration Calorimetry

This is a physical technique, which provides information about the thermodynamic aspects of the interaction between small molecules (NPs) and biomolecules (protein, carbohydrates, DNA *etc.*) The thermodynamics of association between two molecules can be characterized by the association constant (K_a), enthalpy (ΔH_b), free energy (ΔG_b), and entropy (ΔS_b).[82] Many studies have utilized isothermal titration calorimetry (ITC) to determine the thermodynamic properties of protein–NP interactions.[83,84] In this technique, quantification of protein binding on the surface of NPs is achieved by titration of the protein in a solution of the NPs and the heat response is recorded simultaneously, which is then used to get a thermodynamic component measurement with the help of isothermal functions.[85] Huang *et al.*[83] used ITC to study the thermodynamic profile during the interaction of BSA with AuNPs, coated with different agents (negatively charged group, 11-mercapto-1-undecanesulfonate; a mixture of MUS and 1-octanethiol (OT); mixture of MUS and branched OT (3,7-dimethyloctane-1-thiol) (MUS/brOT) called MUS/OT). The ITC data obtained (Figure 1.3) suggested that the adsorption profile of BSA on MUS/OT was different from that of MPA (mercaptopropionic acid)/OT in terms of its heat exchange profile. They observed that the complex of BSA with MUS/brOT was completely exothermic while MUS/OT was initially exothermic but later endothermic and consumed heat for the stabilization of the BSA-AuNP complex. This kind of variation in the thermodynamic profile could be due to a different kind of electrostatic interaction, which takes place because of the difference in the coating agent. Winzen *et al.* utilized ITC to study the affinity of the PCs around hydroxyethyl starch nanocapsules. They found that the presence of a large amount of HSA in the suspension showed a lower binding affinity to the NP surface, which can be attributed to soft

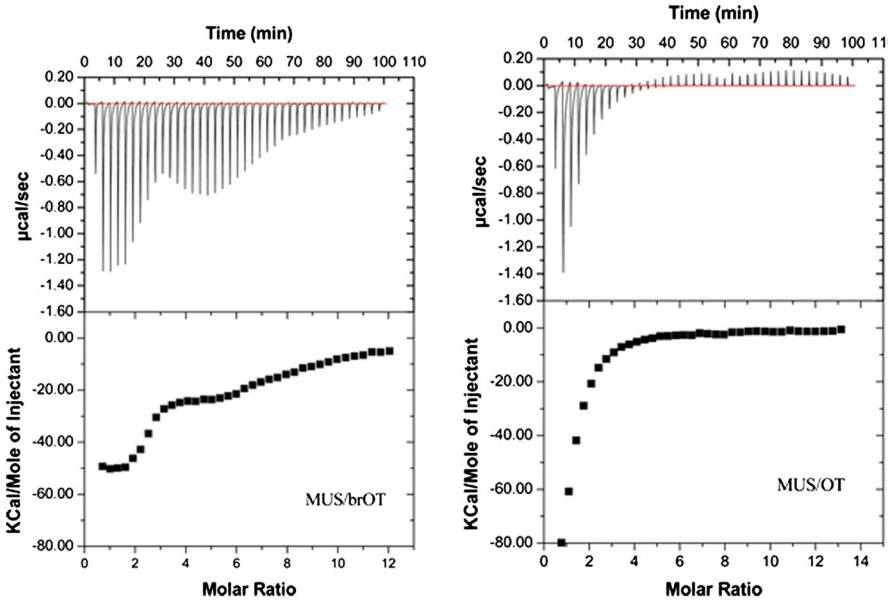


Figure 1.3 Isothermal calorimetric data from the titration of 500 M BSA into 8.4 M MUS/brOT and 7.7 M MUS/OT AuNP. Heat flow *versus* time during injection of proteins at 25 °C and heat evolved per mole of added proteins (corrected for the heat of protein dilution) against the molar ratio (protein–AuNP) for each injection are shown at the top and bottom, respectively. Reproduced from ref. 83 with permission from American Chemical Society, Copyright 2014.

corona formation, whereas the high amount of apolipoprotein A-I offers a greater binding ability due to the hard corona component.[2] Cedervall *et al.* used ITC techniques to examine the effect of different copolymer ratios and different rates of hydrophilicity/hydrophobicity of *N*-isopropylacrylamide (NIPAM)–*N*-*tert*-butylacrylamide (BAM) copolymer (NIPAM–BAM) NPs on the adsorption and desorption of HSA and fibrinogen.[12]

1.5.5 Circular Dichroism Spectroscopy

The structures of proteins vary after adsorption and binding to the surfaces of NPs. The proteins involved in the development of a hard corona generally express the secondary structure such as an α-helix, β-sheets or loops. The secondary structure of proteins in the PC can be obtained by circular dichroism (CD) spectroscopy.[86,87] CD spectroscopy relies on the principle of circularly polarized light that differentiates between the absorption of left- and right-handed polarized lights by proteins. Due to this, the light would have two dissimilar spin angular momentums, which can evaluate the secondary structures of the proteins.[88] Many reports are available that use CD spectroscopy for the determination of protein–NP interactions.[5,89–93] Ahmad *et al.*

have studied the absorption of BSA on the surface of $CoFe_2O_4$ NPs using CD spectroscopy. The results indicated two negative peaks at 208 and 222 nm, which correlates well with to the π–π* and *n*–π* transitions in the α-helix of BSA, respectively. These results further suggested that the interaction of BSA with $CoFe_2O_4$ NPs resulted in a decreased content of α-helix from 55.9% to 48.1% and an enhanced β-sheet content from 37.3% to 39.9%. The secondary structure of the protein has been modified, which suggests the aromatic amino acids are accountable for the binding of proteins to NPs.[93]

1.6 Applications of Protein Coronas

Considering the complexity with the retention of a protein's structure and function, conjugation of the desired protein with a NP becomes extremely challenging. Therefore, covalent or non-covalent binding of the required proteins to NPs leads to the development of novel functional hybrid systems, which offer myriad applications in biomedicines and catalysts. The following sub-topics will summarize the effective strategies developed utilizing protein–corona complex systems for biomedical applications.

1.6.1 Role of the Protein Corona Towards Targeted Drug Delivery

In order to develop an effective targeted drug delivery approach, it is expected that a longer circulation time of NPs in blood and accumulation of drug-loaded NPs at the target site are two of the core criteria. Conventional NPs' surfaces can be modified to increase their stability and prolong the circulation time of nanocarriers along with controlled bio-distribution.[94] A protein covering leads to an alteration of the NPs' hydrodynamic size, shape and, thus, the surface properties. Caracciolo *et al.* reported that after incubation of 3β-[*N*-(*N*′,*N*′-dimethylaminoethane)-carbamoyl]-cholesterol (DCChol)–dioleoylphosphatidylethanolamine DC-Chol-DOPE-DNA lipoplexes with a suspension containing FBS, the size of the NPs was altered from 244 ± 4 nm to 741 ± 1 nm. Therefore, the internalization mechanism of the NPs' entry into cells was also modified from clathrin-dependent to caveolae-mediated.[94] Such events can cause changes in the NPs' cellular internalization and co-localization based on the size. NPs can also be conjugated with different functional groups and moieties for selectively delivering drug(s) to the target site. Transferrin, insulin, folic acid, Apo A-1, and Apo E, *etc.*, are some of the commonly used targeting biomolecules to construct the drug delivery systems targeting cancer tissues. Additional small molecules such as anhydride, amine, hydroxyl, carboxyl, thiol, and epoxy could also be utilized to alter the NP delivery, as they facilitate association of NPs with endothelial cells, pancreatic cancer cells, and activated human macrophages.[95] Some apolipoproteins, such as Apo E, ApoA1, and ApoB-100, are also used to functionalize NPs to target the components of the nervous system.[96,97] The variety

of functional groups present in the PC can be efficiently used for conjugation of the biomolecules mentioned with NPs. In a study, Zensi *et al.* have shown the covalent conjugation of HAS-coated NPs with ApoE, which was intravenously injected into SV 129 mice.[96] They sacrificed the mice after 15–30 mins of treatment and sections of different organs were analysed using TEM (transmission electron microscopy). Interestingly, NPs conjugated with ApoE were only found in the brain capillary endothelial cells and neurons while bare NPs were not taken up by the brain. Surface modifications of NPs can be achieved by the covalent conjugation[98] of NPs with the desired biomolecules. A famous illustration is NP albumin-bound (nab) technology, which consists of binding albumin to a NP's surface to impart effective tumor targeting with concomitantly reduced toxicity. Using this technology, an anticancer agent (abraxane) consisting of paclitaxel was designed. Figure 1.4 demonstrates a novel approach for NP decoration using different surface-modifying ligands specific to cells. Protein adsorption may affect the targeting ligands conjugated on the NP surface; however, several cases showing that functional proteins from the PC could facilitate the recruitment of NPs to the desired target site have been reported. Modification of the NP surface could also induce an alteration in the composition of the PC, which could lead to an interaction between the NP and cell membrane receptors, eventually favouring cellular uptake and other responses of NPs.

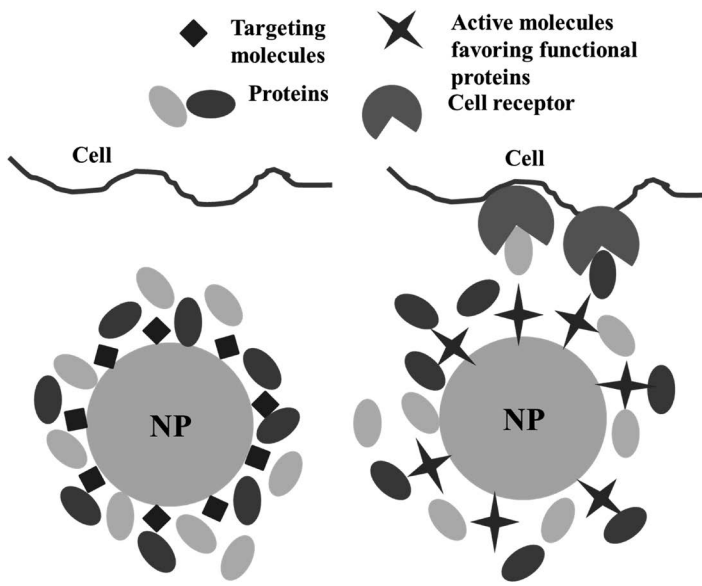


Figure 1.4 Protein-corona-based cell targeting strategy: protein corona formation over functionalized NPs leads to an alteration in the identification of the cell surface receptors (left image); however, protein corona formation along with the active molecules could facilitate the binding of the NPs to the desired targeted cells.

1.6.2 Engineering Nanoparticle–Protein Technologies for Early Cancer Detection

Since PC formation has been reported to alter the properties of NMs, efforts have been devoted to develop technologies that could be used for the sensitive detection of cancer. In this context, several strategies have been developed to probe personalized PCs and their utility in cancer research and management. Mahmoudi *et al.* have investigated the idea of personalized PCs (PPC) in great detail for cancer therapy.[33] PPC have shown a remarkable influence in cancer treatment where the expression pattern of proteins changes as per their physiological environment. Each cancer type is reported to have a variety of characteristic proteins and, therefore, considering the specific interaction between NPs and proteins to form the PC, patients with a specific cancer type may exhibit unique NP coronas. It is well-known that the physio–chemical characteristics of NPs play important roles in the development of PCs around them, therefore, the use of characteristic PCs in the diagnosis of specific diseases would require the synthesis of NPs with the required features.[99] In this context, a detailed understanding of the relationship between the physio–chemical properties of NPs and PC patterns would be the most essential step for developing strategies for diagnostic applications of nanotechnology. The development of PPC was recently validated in pancreatic cancer where clinically relevant proteins were found to be reduced, which affects the size, charge, and aggregation state of NPs.[24,100] PCs developed in pancreatic cancer displayed high negative zeta potentials; however, in other types of cancers, the charge did not vary to any significant magnitude. Thus, the NP and PC technology could work as a "nano-concentrator", which allows identification of the enhancement of a particular protein in the PC developed from cancer patients. In this perspective, the sequestration of proteins across the NP corona surface might be an outstanding opportunity to identify the low-abundant proteins and their connection with carcinogenesis. Another opportunity in which the NP-PC complex could be used as a "nano-constructor'' technology is to diagnose the elevated level of a particular protein in PCs from cancer patients' serum, although their concentration in blood serum is almost similar to that of healthy individuals. Utilizing the "nano-constructor" potential of PCs could be used to identify minor changes in protein concentrations even at the initial stage of disease progression. This strategy could also be used after chemotherapy or surgery, which allows the monitoring of the treatment's success in real time. In this context, blood levels of apolipoproteins could be used as an effective biomarker for the disease's identification, prognosis and to study the development of different types of cancers. The expression profiles of ApoA-1 and ApoC-III in small cell lung cancer cells vary noticeably and, therefore, can be used to distinguish between the non-small cell lung cancer cells from healthy lung tissues.[101] Blood levels of ApoE have been shown to follow the efficiency of chemotherapy as well as the prognosis of several diseases such as Alzheimer's.[102] Thus,

the composition of PPC obtained from leukaemia patients, and its detailed characterization through mass spectroscopy, could be used to develop an appropriate strategy for the early detection of this disease.

1.6.3 Toxicity Reduction

It has been well documented that mammalian cells exposed to NPs undergo an alteration of their cellular physiology and related biochemical and signalling pathways. Despite their dependence on several parameters, NPs may induce toxicity to cells through compromising the membrane integrity of the cell and mitochondria, and frequent impairment of genetic materials. It has also been reported that bare NPs cause more damage to mammalian cells than those capped with biomolecules. Further, the physiological and immunological responses of cells could be altered by PC formation over NPs[103] due to the coating of the surface of NPs with proteins. The formation of a PC on a NP's surface could avoid the damaging effects caused by bare NPs has been investigated in several studies.[104,105] Such results may be due to the formation of a PC that leads to biocompatibility with the human body. BSA adsorption on naked poly-3-hydroxybutyrate-*co*-3-hydroxyhexanoate (PHBHHx NPs) has exhibited less cytotoxicity in comparison to NPs without corona, which was monitored in three different cell lines: A549, HepG2 and L929 cells.[106] Ge *et al.* have studied the spontaneous coating of human blood protein and subsequently followed the toxicity pattern of single-wall carbon nanotubes (SWCNTs) in human acute monocytic leukemia cells (THP-1) and human umbilical vein endothelial cells (HUVECs). The authors observed that bovine fibrinogen (BFG), BSA, transferrin (Tf), and γ globulin (Ig) capping on SWCNTs leads to decreased toxicity compared to bare SWCNT. The SWCNT–protein complex showed binding affinities of proteins in the order of BFG > Ig > Tf > BSA for THP-1 cells.[107] The likely reason behind the minimal toxicity observed by the BFG protein was ascribed to the compact rearrangement of this protein on the SWCNT surface and formation of ~4–5 layers of capping over SWCNT, imparting protection to cells from the direct exposure of SWCNT. Additionally, Žūkienė *et al.* have investigated that BSA coating on ZnO NPs displays less toxicity to CHO cells. It was observed that the binding of BSA to ZnO NPs triggers changes in the structure of BSA during PC formation at 4 °C. However, when corona formation was performed at 20 °C, additional changes in the conformation of BSA were observed (disulfide bridge breakage), which diminished the toxicity reduction effect of BSA-coated ZnO NPs.[108] Tenzer *et al.* have demonstrated that bare silica NPs show a haemolytic effect on red blood cells (RBCs); however, the formation of a PC on the NP surface prevents any damage to RBCs.[15] De paoli *et al.* reported that the interaction between carboxylated-multiwalled carbon nanotubes (CNT-COOH) and platelets is strongly dependent on the types of protein involved in the PC formation. Particularly, a PC composed of the various proteins indicates different influences: albumin PC reduces the platelet aggregation; however, histone H1 and gamma globulins induce platelet aggregation and

fragmentation.[109] Wang *et al.* demonstrated that positively charged amino-modified polystyrene NPs (PS–NH_2–B) cause early cell membrane damage. The PC on the NPs' surface was explained by the "Trojan horse effect" and it was suggested that the NPs were first surrounded by the PC, which gives protection to cell membranes and also prevents intracellular damage until it reaches the lysosome, where the PC undergoes degradation.[104]

1.7 Limitations and Future Perspectives

With the current thrust towards the development of novel NMs for myriad biomedical applications, they are expected to be exposed to a biological milieu where they would interact with suspended proteins. Therefore, the NMs developed must not be considered as bare and the associated properties may not exactly translate in a biological system. Further, it remains to be seen whether the desired physico–chemical properties of NMs are intact even after PC formation, especially the pharmacokinetics and pharmacodynamics. Thus, the possible interactions of NMs with proteins and eventually the formation of the PC must be almost completely characterized. These efforts may include the interaction of proteins with the capping molecules present on the NP surface because the capping ligands offer promising control over their biological interactions. Although such efforts are less complicated than the characterization of a whole protein from the corona, there are limited efforts in this direction. Additionally, such a strategy may offer control over the NMs' interaction with proteins present in biological fluids and subsequently lead to the success of the clinical translation of nanomedicines. Active uptake of NMs is one of the major factors to improve the therapeutic efficacy, therefore, several studies have been performed to compare the uptake pattern. PC formation is also associated with the immune perturbation effect on macrophages. Such events suggest that corona formation could play an important role in potential immune and inflammatory diseases. PC formation may also significantly affect the physiological properties of NMs such as hydrodynamic size, agglomeration behaviour, surface charge, morphology, and sensitivity to pH and the presence of salt. Since all these factors are closely related with the toxicity of NMs, the safety data may also be influenced as a result of PC formation. It has been reported that PC formation over a NM surface leads to the decreased leaching of corresponding ions, thus significant inhibition in particle toxicity. Further, studies revealed that PC modulates the mechanism of uptake of NMs in mammalian cells such as endocytic rates and pathways, transport within the cells, trafficking rate of endosomes and lysosomes and, thus, the overall interaction and effect on the cells. For example, in the case of cell transfection, the presence of serum was found to induce suppression of transfection efficiency due to reduced endo/lysosomal escape of polyplexes with a PC, then PC-free polyplexes. Considering protein as one of the major components of biological fluids, there are other constituent molecules that need to be explored for their interaction with NMs of nanomedicinal potential. Among them, lipids and lipoproteins

must be considered in future for their interactions with NMs and subsequent changes in the physiological properties of NPs and their effect on mammalian cells under *in vitro* and *in vivo* experimental conditions. On the basis of this discussion, it can be concluded that PC formation over a NM surface must be well characterized to realize the full potential of nanotechnology in biomedicines. More components of biological fluids may be considered for their interactions with NMs and subsequent biological effects. Although several efforts have been devoted to addressing these issues, more in-depth studies are needed to completely reveal the PC formation process and composition and translate their effects with biological interactions.

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References

1. Y. Sun and Y. Xia, Shape-controlled synthesis of gold and silver nanoparticles, *Science*, 2002, **298**, 2176–2179.
2. S. Winzen, S. Schoettler, G. Baier, C. Rosenauer, V. Mailaender, K. Landfester and K. Mohr, Complementary analysis of the hard and soft protein corona: sample preparation critically effects corona composition, *Nanoscale*, 2015, **7**, 2992–3001.
3. S. Milani, F. Baldelli Bombelli, A. S. Pitek, K. A. Dawson and J. Rädler, Reversible *versus* irreversible binding of transferrin to polystyrene nanoparticles: soft and hard corona, *ACS Nano*, 2012, **6**, 2532–2541.
4. M. Lundqvist, J. Stigler, G. Elia, I. Lynch, T. Cedervall and K. A. Dawson, Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 14265–14270.
5. W. Liu, J. Rose, S. Plantevin, M. Auffan, J. Y. Bottero and C. Vidaud, Protein corona formation for nanomaterials and proteins of a similar size: hard or soft corona? *Nanoscale*, 2013, **5**, 1658–1668.
6. V. Pareek, A. Bhargava, V. Bhanot, R. Gupta, N. Jain and J. Panwar, Formation and Characterization of Protein Corona Around Nanoparticles: A Review, *J. Nanosci. Nanotechnol.*, 2018, **18**, 6653–6670.

7. T. Cedervall, I. Lynch, M. Foy, T. Berggard, S. C. Donnelly, G. Cagney, S. Linse and K. A. Dawson, Detailed identification of plasma proteins adsorbed on copolymer nanoparticles, *Angew. Chem.*, 2007, **46**, 5754–5756.
8. E. Casals, T. Pfaller, A. Duschl, G. J. Oostingh and V. Puntes, Time evolution of the nanoparticle protein corona, *ACS Nano*, 2010, **4**, 3623–3632.
9. D. Dell'Orco, M. Lundqvist, C. Oslakovic, T. Cedervall and S. Linse, Modeling the time evolution of the nanoparticle-protein corona in a body fluid, *PLoS One*, 2010, **5**, e10949.
10. C. Röcker, M. Pötzl, F. Zhang, W. J. Parak and G. U. Nienhaus, A quantitative fluorescence study of protein monolayer formation on colloidal nanoparticles, *Nat. Nanotechnol.*, 2009, **4**, 577.
11. E. A. Vogler, Protein adsorption in three dimensions, *Biomaterials*, 2012, **33**, 1201–1237.
12. T. Cedervall, I. Lynch, S. Lindman, T. Berggård, E. Thulin, H. Nilsson, K. A. Dawson and S. Linse, Understanding the nanoparticle–protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 2050–2055.
13. I. Delfino and S. Cannistraro, Optical investigation of the electron transfer protein azurin–gold nanoparticle system, *Biophys. Chem.*, 2009, **139**, 1–7.
14. Z. J. Deng, G. Mortimer, T. Schiller, A. Musumeci, D. Martin and R. F. Minchin, Differential plasma protein binding to metal oxide nanoparticles, *Nanotechnology*, 2009, **20**, 455101.
15. S. Tenzer, D. Docter, S. Rosfa, A. Wlodarski, J. Kuharev, A. Rekik, S. K. Knauer, C. Bantz, T. Nawroth, C. Bier, J. Sirirattanapan, W. Mann, L. Treuel, R. Zellner, M. Maskos, H. Schild and R. H. Stauber, Nanoparticle size is a critical physicochemical determinant of the human blood plasma corona: a comprehensive quantitative proteomic analysis, *ACS Nano*, 2011, **5**, 7155–7167.
16. M. P. Monopoli, C. Aberg, A. Salvati and K. A. Dawson, Biomolecular coronas provide the biological identity of nanosized materials, *Nat. Nanotechnol.*, 2012, **7**, 779–786.
17. C. D. Walkey and W. C. Chan, Understanding and controlling the interaction of nanomaterials with proteins in a physiological environment, *Chem. Soc. Rev.*, 2012, **41**, 2780–2799.
18. D. Docter, D. Westmeier, M. Markiewicz, S. Stolte, S. K. Knauer and R. H. Stauber, The nanoparticle biomolecule corona: lessons learned - challenge accepted? *Chem. Soc. Rev.*, 2015, **44**, 6094–6121.
19. C. Sacchetti, K. Motamedchaboki, A. Magrini, G. Palmieri, M. Mattei, S. Bernardini, N. Rosato, N. Bottini and M. Bottini, Surface polyethylene glycol conformation influences the protein corona of polyethylene glycol-modified single-walled carbon nanotubes: potential implications on biological performance, *ACS Nano*, 2013, **7**, 1974–1989.
20. A. L. Capriotti, G. Caracciolo, G. Caruso, P. Foglia, D. Pozzi, R. Samperi and A. Lagana, Differential analysis of "protein corona" profile adsorbed onto different nonviral gene delivery systems, *Anal. Biochem.*, 2011, **419**, 180–189.

21. D. Walczyk, F. B. Bombelli, M. P. Monopoli, I. Lynch and K. A. Dawson, What the cell "sees" in bionanoscience, *J. Am. Chem. Soc.*, 2010, **132**, 5761–5768.
22. H. D. Hill, J. E. Millstone, M. J. Banholzer and C. A. Mirkin, The role radius of curvature plays in thiolated oligonucleotide loading on gold nanoparticles, *ACS Nano*, 2009, **3**, 418–424.
23. M. Mahmoudi, I. Lynch, M. R. Ejtehadi, M. P. Monopoli, F. B. Bombelli and S. Laurent, Protein-nanoparticle interactions: opportunities and challenges, *Chem. Rev.*, 2011, **111**, 5610–5637.
24. M. A. Dobrovolskaia, A. K. Patri, J. Zheng, J. D. Clogston, N. Ayub, P. Aggarwal, B. W. Neun, J. B. Hall and S. E. McNeil, Interaction of colloidal gold nanoparticles with human blood: effects on particle size and analysis of plasma protein binding profiles, *Nanomedicine: Nanotechnol., Biol. Med.*, 2009, **5**, 106–117.
25. J. Piella, N. G. Bastus and V. Puntes, Size-Dependent Protein-Nanoparticle Interactions in Citrate-Stabilized Gold Nanoparticles: The Emergence of the Protein Corona, *Bioconjugate Chem.*, 2017, **28**, 88–97.
26. R. García-Álvarez, M. Hadjidemetriou, A. Sánchez-Iglesias, L. M. Liz-Marzán and K. Kostarelos, *In vivo* formation of protein corona on gold nanoparticles. The effect of their size and shape, *Nanoscale*, 2018, **10**, 1256–1264.
27. P. Foroozandeh and A. A. Aziz, Merging worlds of nanomaterials and biological environment: factors governing protein corona formation on nanoparticles and its biological consequences, *Nanoscale Res. Lett.*, 2015, **10**, 221.
28. Z. Ma, J. Bai, Y. Wang and X. Jiang, Impact of shape and pore size of mesoporous silica nanoparticles on serum protein adsorption and RBCs hemolysis, *ACS Appl. Mater. Interfaces*, 2014, **6**, 2431–2438.
29. P. Roach, D. Farrar and C. C. Perry, Interpretation of protein adsorption: surface-induced conformational changes, *J. Am. Chem. Soc.*, 2005, **127**, 8168–8173.
30. A. Gessner, R. Waicz, A. Lieske, B. Paulke, K. Mader and R. H. Muller, Nanoparticles with decreasing surface hydrophobicities: influence on plasma protein adsorption, *Int. J. Pharm.*, 2000, **196**, 245–249.
31. S. Dominguez-Medina, J. Blankenburg, J. Olson, C. F. Landes and S. Link, Adsorption of a Protein Monolayer *via* Hydrophobic Interactions Prevents Nanoparticle Aggregation under Harsh Environmental Conditions, *ACS Sustainable Chem. Eng.*, 2013, **1**, 833–842.
32. M. Hulander, A. Lundgren, M. Berglin, M. Ohrlander, J. Lausmaa and H. Elwing, Immune complement activation is attenuated by surface nanotopography, *Int. J. Nanomed.*, 2011, **6**, 2653–2666.
33. M. Mahmoudi, M. A. Sahraian, M. A. Shokrgozar and S. Laurent, Superparamagnetic iron oxide nanoparticles: promises for diagnosis and treatment of multiple sclerosis, *ACS Chem. Neurosci.*, 2011, **2**, 118–140.
34. M. S. Ehrenberg, A. E. Friedman, J. N. Finkelstein, G. Oberdorster and J. L. McGrath, The influence of protein adsorption on nanoparticle association with cultured endothelial cells, *Biomaterials*, 2009, **30**, 603–610.

35. I. Lynch, A. Salvati and K. A. Dawson, Protein-nanoparticle interactions: What does the cell see? *Nat. Nanotechnol.*, 2009, **4**, 546–547.
36. A. Gessner, A. Lieske, B. Paulke and R. Muller, Influence of surface charge density on protein adsorption on polymeric nanoparticles: analysis by two-dimensional electrophoresis, *Eur. J. Pharm. Biopharm.*, 2002, **54**, 165–170.
37. A. S. Fauci, *Harrison's Principles of Internal Medicine*, Mcgraw-hill, New York, 1998.
38. J. M. Yang, H. Yang and L. Lin, Quantum dot nano thermometers reveal heterogeneous local thermogenesis in living cells, *ACS Nano*, 2011, **5**, 5067–5071.
39. J. D. Hasday and I. S. Singh, Fever and the heat shock response: distinct, partially overlapping processes, *Cell Stress Chaperones*, 2000, **5**, 471–480.
40. M. Mahmoudi, A. M. Abdelmonem, S. Behzadi, J. H. Clement, S. Dutz, M. R. Ejtehadi, R. Hartmann, K. Kantner, U. Linne and P. Maffre, Temperature: the "ignored" factor at the nanobio interface, *ACS Nano*, 2013, **7**, 6555–6562.
41. M. Mahmoudi, S. E. Lohse, C. J. Murphy, A. Fathizadeh, A. Montazeri and K. S. Suslick, Variation of protein corona composition of gold nanoparticles following plasmonic heating, *Nano Lett.*, 2014, **14**, 6–12.
42. J. O'Brien and K. J. Shea, Tuning the Protein Corona of Hydrogel Nanoparticles: The Synthesis of Abiotic Protein and Peptide Affinity Reagents, *Acc. Chem. Res.*, 2016, **49**, 1200–1210.
43. V. H. Nguyen and B. J. Lee, Protein corona: a new approach for nanomedicine design, *Int. J. Nanomed.*, 2017, **12**, 3137–3151.
44. M. Kosmulski, pH-dependent surface charging and points of zero charge. IV. Update and new approach, *J. Colloid Interface Sci.*, 2009, **337**, 439–448.
45. G. Chen, X. Liu and C. Su, Distinct effects of humic acid on transport and retention of TiO2 rutile nanoparticles in saturated sand columns, *Environ. Sci. Technol.*, 2012, **46**, 7142–7150.
46. K. Pakarinen, E. J. Petersen, L. Alvila, G. C. Waissi-Leinonen, J. Akkanen, M. T. Leppanen and J. V. Kukkonen, A screening study on the fate of fullerenes (nC60) and their toxic implications in natural freshwaters, *Environ. Toxicol. Chem.*, 2013, **32**, 1224–1232.
47. B. Xie, Z. Xu, W. Guo and Q. Li, Impact of natural organic matter on the physicochemical properties of aqueous C60 nanoparticles, *Environ. Sci. Technol.*, 2008, **42**, 2853–2859.
48. A. B. Engin, M. Neagu, K. Golokhvast and A. Tsatsakis, Nanoparticles and endothelium: an update on the toxicological interactions, *Farmacia*, 2015, **63**, 792–804.
49. R. Gref, M. Luck, P. Quellec, M. Marchand, E. Dellacherie, S. Harnisch, T. Blunk and R. H. Muller, 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption, *Colloids Surf., B*, 2000, **18**, 301–313.

50. J. L. Perry, K. G. Reuter, M. P. Kai, K. P. Herlihy, S. W. Jones, J. C. Luft, M. Napier, J. E. Bear and J. M. DeSimone, PEGylated PRINT Nanoparticles: The Impact of PEG Density on Protein Binding, Macrophage Association, Biodistribution, and Pharmacokinetics, *Nano Lett.*, 2012, **12**, 5304–5310.
51. S. M. Moghimi, I. Muir, L. Illum, S. S. Davis and V. Kolb-Bachofen, Coating particles with a block co-polymer (poloxamine-908) suppresses opsonization but permits the activity of dysopsonins in the serum, *Biochim. Biophys. Acta*, 1993, **1179**, 157–165.
52. P. Aggarwal, J. B. Hall, C. B. McLeland, M. A. Dobrovolskaia and S. E. McNeil, Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy, *Adv. Drug Delivery Rev.*, 2009, **61**, 428–437.
53. D. Dutta, S. K. Sundaram, J. G. Teeguarden, B. J. Riley, L. S. Fifield, J. M. Jacobs, S. R. Addleman, G. A. Kaysen, B. M. Moudgil and T. J. Weber, Adsorbed proteins influence the biological activity and molecular targeting of nanomaterials, *Toxicol. Sci.*, 2007, **100**, 303–315.
54. L. K. Muller, J. Simon, C. Rosenauer, V. Mailander, S. Morsbach and K. Landfester, The Transferability from Animal Models to Humans: Challenges Regarding Aggregation and Protein Corona Formation of Nanoparticles, *Biomacromolecules*, 2018, **19**, 374–385.
55. M. P. Monopoli, D. Walczyk, A. Campbell, G. Elia, I. Lynch, F. B. Bombelli and K. A. Dawson, Physical-chemical aspects of protein corona: relevance to *in vitro* and *in vivo* biological impacts of nanoparticles, *J. Am. Chem. Soc.*, 2011, **133**, 2525–2534.
56. U. Sakulkhu, M. Mahmoudi, L. Maurizi, J. Salaklang and H. Hofmann, Protein corona composition of superparamagnetic iron oxide nanoparticles with various physico-chemical properties and coatings, *Sci. Rep.*, 2014, **4**, 5020.
57. C. C. Fleischer and C. K. Payne, Nanoparticle-cell interactions: molecular structure of the protein corona and cellular outcomes, *Acc. Chem. Res.*, 2014, **47**, 2651–2659.
58. P. P. Karmali and D. Simberg, Interactions of nanoparticles with plasma proteins: implication on clearance and toxicity of drug delivery systems, *Expert Opin. Drug Delivery*, 2011, **8**, 343–357.
59. S. R. Saptarshi, A. Duschl and A. L. Lopata, Interaction of nanoparticles with proteins: relation to bio-reactivity of the nanoparticle, *J. Nanobiotechnol.*, 2013, **11**, 26.
60. J. H. Shannahan, X. Lai, P. C. Ke, R. Podila, J. M. Brown and F. A. Witzmann, Silver nanoparticle protein corona composition in cell culture media, *PLoS One*, 2013, **8**, e74001.
61. K. Sempf, T. Arrey, S. Gelperina, T. Schorge, B. Meyer, M. Karas and J. Kreuter, Adsorption of plasma proteins on uncoated PLGA nanoparticles, *Eur. J. Pharm. Biopharm.*, 2013, **85**, 53–60.
62. F. Darabi Sahneh, C. Scoglio and J. Riviere, Dynamics of nanoparticle-protein corona complex formation: analytical results from population balance equations, *PLoS One*, 2013, **8**, e64690.

63. L. Vroman, A. L. Adams, G. C. Fischer and P. C. Munoz, Interaction of high molecular weight kininogen, factor XII, and fibrinogen in plasma at interfaces, *Blood*, 1980, **55**, 156–159.
64. T. M. Goppert and R. H. Muller, Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for intravenous targeting of drugs to the brain: comparison of plasma protein adsorption patterns, *J. Drug Targeting*, 2005, **13**, 179–187.
65. M. Jansch, P. Stumpf, C. Graf, E. Ruhl and R. H. Muller, Adsorption kinetics of plasma proteins on ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles, *Int. J. Pharm.*, 2012, **428**, 125–133.
66. S. C. Gill and P. H. von Hippel, Calculation of protein extinction coefficients from amino acid sequence data, *Anal. Biochem.*, 1989, **182**, 319–326.
67. N. Jain, A. Bhargava, M. Rathi, R. V. Dilip and J. Panwar, Removal of Protein Capping Enhances the Antibacterial Efficiency of Biosynthesized Silver Nanoparticles, *PLoS One*, 2015, **10**, e0134337.
68. I. Yadav, V. K. Aswal and J. Kohlbrecher, Size-dependent interaction of silica nanoparticles with lysozyme and bovine serum albumin proteins, *Phys. Rev. E*, 2016, **93**, 052601.
69. X. Xu, Y. Wang, H. Wang, H. Su, X. Mao, L. Jiang, M. Liu, D. Sun and S. Hou, Synthesis of triangular silver nanoprisms and studies on the interactions with human serum albumin, *J. Mol. Liq.*, 2016, **220**, 14–20.
70. N. Dasgupta, S. Ranjan, D. Patra, P. Srivastava, A. Kumar and C. Ramalingam, Bovine serum albumin interacts with silver nanoparticles with a "side-on" or "end on" conformation, *Chem.-Biol. Interact.*, 2016, **253**, 100–111.
71. R. Singh and S. Singh, Role of phosphate on stability and catalase mimetic activity of cerium oxide nanoparticles, *Colloids Surf., B*, 2015, **132**, 78–84.
72. J. Vidic, F. Haque, J. M. Guigner, A. Vidy, C. Chevalier and S. Stankic, Effects of water and cell culture media on the physicochemical properties of ZnMgO nanoparticles and their toxicity toward mammalian cells, *Langmuir*, 2014, **30**, 11366–11374.
73. J. Kong and S. Yu, Fourier transform infrared spectroscopic analysis of protein secondary structures, *Acta Biochim. Biophys. Sin.*, 2007, **39**, 549–559.
74. D. A. Mbeh, T. Javanbakht, L. Tabet, Y. Merhi, K. Maghni, E. Sacher and L. H. Yahia, Protein Corona Formation on Magnetite Nanoparticles: Effects of Culture Medium Composition, and Its Consequences on Superparamagnetic Nanoparticle Cytotoxicity, *J. Biomed. Nanotechnol.*, 2015, **11**, 828–840.
75. L. Fageria, V. Pareek, R. V. Dilip, A. Bhargava, S. S. Pasha, I. R. Laskar, H. Saini, S. Dash, R. Chowdhury and J. Panwar, Biosynthesized protein-capped silver nanoparticles induce ROS-dependent proapoptotic signals and prosurvival autophagy in cancer cells, *ACS Omega*, 2017, **2**, 1489–1504.

76. L. Shang, Y. Wang, J. Jiang and S. Dong, pH-dependent protein conformational changes in albumin:gold nanoparticle bioconjugates: a spectroscopic study, *Langmuir*, 2007, **23**, 2714–2721.
77. W. Goldburg, Dynamic light scattering, *Am. J. Phys.*, 1999, **67**, 1152–1160.
78. P. M. Tessier, J. Jinkoji, Y.-C. Cheng, J. L. Prentice and A. M. Lenhoff, Self-interaction nanoparticle spectroscopy: a nanoparticle-based protein interaction assay, *J. Am. Chem. Soc.*, 2008, **130**, 3106–3112.
79. P. Del Pino, B. Pelaz, Q. Zhang, P. Maffre, G. U. Nienhaus and W. J. Parak, Protein corona formation around nanoparticles–from the past to the future, *Mater. Horiz.*, 2014, **1**, 301–313.
80. S. Patil, A. Sandberg, E. Heckert, W. Self and S. Seal, Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential, *Biomaterials*, 2007, **28**, 4600–4607.
81. K. Natte, J. F. Friedrich, S. Wohlrab, J. Lutzki, R. von Klitzing, W. Osterle and G. Orts-Gil, Impact of polymer shell on the formation and time evolution of nanoparticle-protein corona, *Colloids Surf., B*, 2013, **104**, 213–220.
82. M. M. Pierce, C. Raman and B. T. Nall, Isothermal titration calorimetry of protein–protein interactions, *Methods*, 1999, **19**, 213–221.
83. R. Huang, R. P. Carney, K. Ikuma, F. Stellacci and B. L. Lau, Effects of surface compositional and structural heterogeneity on nanoparticle-protein interactions: different protein configurations, *ACS Nano*, 2014, **8**, 5402–5412.
84. M. De, C. C. You, S. Srivastava and V. M. Rotello, Biomimetic interactions of proteins with functionalized nanoparticles: a thermodynamic study, *J. Am. Chem. Soc.*, 2007, **129**, 10747–10753.
85. Q. Xiao, S. Huang, Z. D. Qi, B. Zhou, Z. K. He and Y. Liu, Conformation, thermodynamics and stoichiometry of HSA adsorbed to colloidal CdSe/ZnS quantum dots, *Biochim. Biophys. Acta*, 2008, **1784**, 1020–1027.
86. N. J. Greenfield, Using circular dichroism spectra to estimate protein secondary structure, *Nat. Protoc.*, 2006, **1**, 2876–2890.
87. N. Sreerama, S. Y. Venyaminov and R. W. Woody, Estimation of protein secondary structure from circular dichroism spectra: inclusion of denatured proteins with native proteins in the analysis, *Anal. Biochem.*, 2000, **287**, 243–251.
88. W. C. Johnson Jr, Secondary structure of proteins through circular dichroism spectroscopy, *Annu. Rev. Biophys. Biophys. Chem.*, 1988, **17**, 145–166.
89. M. Lundqvist, I. Sethson and B. H. Jonsson, Protein adsorption onto silica nanoparticles: conformational changes depend on the particles' curvature and the protein stability, *Langmuir*, 2004, **20**, 10639–10647.
90. S. H. Lacerda, J. J. Park, C. Meuse, D. Pristinski, M. L. Becker, A. Karim and J. F. Douglas, Interaction of gold nanoparticles with common human blood proteins, *ACS Nano*, 2010, **4**, 365–379.
91. L. Li, Q. Mu, B. Zhang and B. Yan, Analytical strategies for detecting nanoparticle-protein interactions, *Analyst*, 2010, **135**, 1519–1530.

92. G. Brancolini, D. B. Kokh, L. Calzolai, R. C. Wade and S. Corni, Docking of ubiquitin to gold nanoparticles, *ACS Nano*, 2012, **6**, 9863–9878.
93. F. Ahmad, Y. Zhou, Z. Ling, Q. Xiang and X. Zhou, Systematic elucidation of interactive unfolding and corona formation of bovine serum albumin with cobalt ferrite nanoparticles, *RSC Adv.*, 2016, **6**, 35719–35730.
94. G. Caracciolo, L. Callipo, S. C. De Sanctis, C. Cavaliere, D. Pozzi and A. Lagana, Surface adsorption of protein corona controls the cell internalization mechanism of DC-Chol-DOPE/DNA lipoplexes in serum, *Biochim. Biophys. Acta*, 2010, **1798**, 536–543.
95. R. Weissleder, K. Kelly, E. Y. Sun, T. Shtatland and L. Josephson, Cell-specific targeting of nanoparticles by multivalent attachment of small molecules, *Nat. Biotechnol.*, 2005, **23**, 1418.
96. A. Zensi, D. Begley, C. Pontikis, C. Legros, L. Mihoreanu, S. Wagner, C. Büchel and J. Kreuter, Albumin nanoparticles targeted with Apo E enter the CNS by transcytosis and are delivered to neurones, *J. Controlled Release*, 2009, **137**, 78–86.
97. J. Kreuter, T. Hekmatara, S. Dreis, T. Vogel, S. Gelperina and K. Langer, Covalent attachment of apolipoprotein AI and apolipoprotein B-100 to albumin nanoparticles enables drug transport into the brain, *J. Controlled Release*, 2007, **118**, 54–58.
98. L. Thiele, J. E. Diederichs, R. Reszka, H. P. Merkle and E. Walter, Competitive adsorption of serum proteins at microparticles affects phagocytosis by dendritic cells, *Biomaterials*, 2003, **24**, 1409–1418.
99. S. Palchetti, D. Pozzi, M. Mahmoudi and G. Caracciolo, Exploitation of nanoparticle–protein corona for emerging therapeutic and diagnostic applications, *J. Mater. Chem. B*, 2016, **4**, 4376–4381.
100. V. Colapicchioni, M. Tilio, L. Digiacomo, V. Gambini, S. Palchetti, C. Marchini, D. Pozzi, S. Occhipinti, A. Amici and G. Caracciolo, Personalized liposome-protein corona in the blood of breast, gastric and pancreatic cancer patients, *Int. J. Biochem. Cell Biol.*, 2016, **75**, 180–187.
101. J. Shi, H. Yang, X. Duan, L. Li, L. Sun, Q. Li and J. Zhang, Apolipoproteins as Differentiating and Predictive Markers for Assessing Clinical Outcomes in Patients with Small Cell Lung Cancer, *Yonsei Med. J.*, 2016, **57**, 549–556.
102. F. J. Wolters, P. J. Koudstaal, A. Hofman, C. M. van Duijn and M. A. Ikram, Serum apolipoprotein E is associated with long-term risk of Alzheimer's disease: The Rotterdam Study, *Neurosci. Lett.*, 2016, **617**, 139–142.
103. Y. K. Lee, E. J. Choi, T. J. Webster, S. H. Kim and D. Khang, Effect of the protein corona on nanoparticles for modulating cytotoxicity and immunotoxicity, *Int. J. Nanomed.*, 2015, **10**, 97–113.
104. F. Wang, L. Yu, M. P. Monopoli, P. Sandin, E. Mahon, A. Salvati and K. A. Dawson, The biomolecular corona is retained during nanoparticle uptake and protects the cells from the damage induced by cationic nanoparticles until degraded in the lysosomes, *Nanomedicine: Nanotechnol., Biol. Med.*, 2013, **9**, 1159–1168.

105. R. Tedja, M. Lim, R. Amal and C. Marquis, Effects of serum adsorption on cellular uptake profile and consequent impact of titanium dioxide nanoparticles on human lung cell lines, *ACS Nano*, 2012, **6**, 4083–4093.
106. Q. Peng, S. Zhang, Q. Yang, T. Zhang, X.-Q. Wei, L. Jiang, C.-L. Zhang, Q.-M. Chen, Z.-R. Zhang and Y.-F. Lin, Preformed albumin corona, a protective coating for nanoparticles based drug delivery system, *Biomaterials*, 2013, **34**, 8521–8530.
107. C. Ge, J. Du, L. Zhao, L. Wang, Y. Liu, D. Li, Y. Yang, R. Zhou, Y. Zhao and Z. Chai, Binding of blood proteins to carbon nanotubes reduces cytotoxicity, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, 16968–16973.
108. R. Žūkienė and V. Snitka, Zinc oxide nanoparticle and bovine serum albumin interaction and nanoparticles influence on cytotoxicity *in vitro*, *Colloids Surf., B*, 2015, **135**, 316–323.
109. S. H. De Paoli, L. L. Diduch, T. Z. Tegegn, M. Orecna, M. B. Strader, E. Karnaukhova, J. E. Bonevich, K. Holada and J. Simak, The effect of protein corona composition on the interaction of carbon nanotubes with human blood platelets, *Biomaterials*, 2014, **35**, 6182–6194.