CHAPTER I
INTRODUCTION
1.1 Nanoparticle-Protein Interaction

The investigation of nanoparticle-protein (NP-protein) interaction is crucial for the development of novel biomaterials and hierarchically assembled nanoarchitectures, which offer many advantages in the field of biomedical, sensing, and energy applications.\textsuperscript{1-5} Understanding NP-protein interaction is pivotal to probe how NPs target specific cells as these proteins control the specific cellular receptors used for the formation of NP-protein complex,\textsuperscript{6-8} the cellular internalization pathway,\textsuperscript{9,10} and even the immune response.\textsuperscript{11-14} These NP-protein interactions influence the cellular-level events that include NP binding, internalization and transport, where protein adsorption on nanoparticles (NPs) is mediated by van der Waals, H-bonds, electrostatic, hydrophobic, and π−π stacking interactions. The complexity of the interaction between proteins and NPs remain challenge since the adsorbed proteins control the interaction of NPs with cells.\textsuperscript{15} It may be appeared that protein interactions with a nanoscaled surface may lead to the “cryptic” peptide epitopes, resulting in a change in protein conformation and activity loss.\textsuperscript{16-20} The fibrillation of proteins and peptides accelerated by NPs is associated with several protein misfolding diseases such as Alzheimer’s and Parkinson’s diseases.\textsuperscript{21} In contrast, protein adsorption to NP surface could also alter the properties of NPs giving it a biological identity that is distinct from its synthetic identity as shown in Figure 1.1.\textsuperscript{15} It is the biological identity that decides the physiological response of NPs by mediating their interaction with proteins, which is a function of NP’s synthetic identity such as size, shape, and surface chemistry, environment, and exposure time. In particular, biological identity of the NP-protein complex might differ from that of individual protein and NPs. For example, hydrodynamic diameter of graphene oxide (GO, 100-500 nm) and serum proteins are too small to get trapped by pulmonary capillary (capillary diameter ∼ 7 μm).\textsuperscript{22} On the other hand, the protein–GO
complex is filtered by the pulmonary capillary due to the formation of large agglomerates and hence, trapped in the lungs. The targeted specificity of transferrin-conjugated NPs was lost upon exposure to biological serum, which prevents transferrin from binding to its targeted receptors on the cell surface. Therefore, it is worthwhile to understand the structure of protein adsorbed on the NP surface in NP-protein complex which preserves protein activity and conformation thereby providing basis to fabricate NPs for their potential application in the field of biology and medicine.\textsuperscript{2-3, 24}

\textbf{Figure 1.1} Exposure of NPs into physiological environment acquires biological identity by the formation of corona. Hard corona binds tightly to NP surface (green) with high affinity and soft corona (red) with low affinity experiences reversible adsorption over NP surface.

\textbf{1.2 The basis of protein corona formation}

The dynamic layer of protein formed at the NP-protein interface when NPs met with smorgasbord of extracellular protein\textsuperscript{25-27} forms “corona” on the NP surface, which is responsible for the different cellular responses.\textsuperscript{2, 28} Thus, the corona formed is the effective unit of interest in the nanomaterial–cell interaction, which programmes the
biological fate and function of NPs by physiological response. The cell ‘sees’ and interacts with the entire NP–protein corona (NP-PC) complexes rather than with the ‘bare’ entity of NP. Protein binding affinities and specificities are encoded by the composition of the corona, which depend on NP size and surface characteristics.\textsuperscript{3,29}

Based on the affinity of proteins towards NP surface, corona formation can be classified into hard corona and soft corona. Irreversible binding of proteins to the NPs leads to the formation of “hard corona” where proteins form stable hard core over particles that possess strongly affinity for the NP surface with longer surface residence time and are in higher abundance amongst proteins population in corona (Figure 1.2). Plasma proteins such as immunoglobulins and apolipoproteins form hard corona with various types of NPs.\textsuperscript{30} Proteins that possess weak association to the particles with quick reversible binding to NP surface defined as “soft corona” resides for shorter time with lower abundance in the corona compared to the other members of protein corona as shown in Figure 1.2. Those weakly bound proteins exchange at faster rates with free proteins, which is anticipated by their rapid dissociation upon decreasing the concentration of free proteins in the biological medium.\textsuperscript{31} Corona formation leads to the development of an “adsorbome” consisting of 166 plasma proteins over NP surfaces.\textsuperscript{32} However, the major proteins in the plasma are present at relatively low concentration in the corona than less abundant proteins.\textsuperscript{33} For example, inspite of their richness in plasma proteins such as transferrin and albumin, they are detected in very low concentrations in the hard corona. On the contrary, higher composition of lower abundant proteins such as immunoglobulins, apolipoproteins, and fibrinogen are detected in the hard corona.\textsuperscript{34-36} Consequently, due to the specific interaction with less abundant proteins, NPs act as concentrator for proteins, which lead to significant difference in corona formation.
Figure 1.2 Representation of protein corona formation on a nanoparticle (NP) surface. Protein adsorptions are kinetic (k) and thermodynamic (K) functions of both the individual proteins and NP properties.

1.2.1 Factors contributing to corona formation

1.2.1.1 Dynamic nature of protein corona

Current development in PC formation affords biological identity to NP-protein complex by spontaneous coating of protein over pristine NPs surface. Such corona formation involves fast conjugation dynamics where within fraction of time NPs are coated by proteins, which are found to be transient moving towards stable configuration.\textsuperscript{28, 37-38}

The dynamic nature of protein corona is governed by wide variety of factors such as variation in binding affinity of proteins leading to different residence time of proteins over NPs surface, disparity in protein profiles of the biological medium and particle-protein association/dissociation rate.\textsuperscript{3, 39} Exposure of NPs to bloodstream results in the competitive adsorption of proteins present in plasma. The high mobility proteins adsorbs first over NPs surface which is subsequently replaced by less motile, high
affinity proteins by slow exchange kinetics known as vroman effect. This may take up few hours to several days with long residence time to attain equilibrium leading to the formation of hard corona. Though equilibrium is associated with the partial desorption of the NPs associated proteins, NP-protein composition remains stable in protein-free media (hard protein corona), which is called as protein crowding effects at the NP surface. The dynamic nature of NP-protein complex was elucidated by the interaction of citrate stabilized AuNPs in cell culture medium containing bovine serum albumin. The time evolution of corona formation was attributed to discrepancy in hydrodynamic size, surface plasmon resonance band and surface charge of NPs. For shorter incubation time (minutes), the properties of NPs can be recovered. In contrast, the change in property of NPs was found to be eternal indicating the transition from weak soft corona to stable hard corona for longer incubation time (48h). Time evolution of corona composition upon exposure of mannan (polymer of mannose) nanogel to human plasma implied that apolipoproteins B100 and A-I to be dominant corona proteins for 1h incubation whereas HSA predominates at longer incubation time of 24h. Dynamic nature of protein corona formation by variety of nanomaterials with similar hydrophobicity and surface charge indicated the formation of hard corona over 48h of incubation. However, decrease in production of reactive oxygen species was observed with prolonged incubation time. Therefore, different residence time of NPs in biological environment affects NP-protein equilibrium, which in turn determines NPs identity and its biological interaction. Exposure of NPs to human plasma detected 166 proteins in corona at the earlier time, which extended to 300 proteins on prolonged plasma exposure time where corona composition changed only quantitatively and not qualitatively. In addition to the plasma exposure time, corona composition is also
affected by intrinsic properties of NPs such as size\textsuperscript{45-50} shape and surface charge,\textsuperscript{51-52} and surface functionalization,\textsuperscript{53-55} and hydrophobicity\textsuperscript{30,35} (Figure 1.3).

**Figure 1.3** Schematic representation of factors influencing NP-protein interactions

### 1.2.1.2 NP state

The impact of surface chemistry on corona formation can be studied by the modification of NP surface with various functional groups. PC formation and its composition with Silica (SiO\textsubscript{2}) NPs possessing different surface chemistry such as amine and carboxyl groups were studied. Mass spectrometry demonstrated transition from dynamic corona to more stable corona with respect to time. Agglomeration rate was found to be slow in the case of carboxyl functionalization compared to that of bare and amine terminal. Furthermore, toxicity of NPs was dictated by lactate dehydrogenase release and tetrazolium reduction, respectively, in mouse alveolar macrophages and mouse lung epithelial cells.\textsuperscript{56} The influence of hydrophobicity of ligands on the corona formation was examined by flow field-flow fractionation and ultracentrifugation, which rapidly differentiated the corona proteins based on their
exchange rates. More hydrophobic the ligand, more dynamic corona formed which involves binding of proteins with higher surface hydrophobicity assisted by increase in the surface hydrophobicity of the SPIONs (Super paramagnetic iron oxide nanoparticles) with a core size around 10 nm. Cellular uptake of one of the corona protein, transferrin enhanced uptake in the case of stable corona while dynamic corona exhibits trivial impact. The composition of protein corona on core-shell SPION with different surface coated polyvinyl alcohol polymer (PVA) (positive, neutral and negative), SiO$_2$ (positive and negative), titanium dioxide and metal gold assessed the impact of surface charge and surface material on the corona composition. The orientation of ligands on NP surface possessed significant impact on the NP-protein interaction. Corona formation on PEG (2kda) modified SWCNT (single walled carbon nanotube) were dependent on PEG orientation and surface charge. Immunoglobulins and apolipoproteins identified as corona proteins are independent of molecular weight, isoelectric point, total hydrophobicity, and number of polyaromatic residues of the proteins. The transition from mushroom to mushroom-brush conformation of PEG over NPs surface influenced the adsorption of protein over PEG-SWCNT that facilitate shorter blood circulation time, faster renal excretion, and higher relative spleen versus liver uptake of PEG-SWCNTs. Thus, it is apparent that the stability, structure and function of NP-PC is dictated by surface chemistry of NPs.

1.2.1.3 NP size and charge

Though surface functionalization of NPs contributes to the PC formation, the role of NP size and charge is inevitable. Hence, PC formation over engineered NPs with different size and surface charge was investigated. Functionalised NPs with different size were probed to evaluate corona formation using 1D PAGE and LC-MS/MS upon incubation
of NPs with plasma for 1h. Though unmodified PSNPs (polystyrene nanoparticles) with different size possess similar PC composition, the modified PSNPs with various size differ in corona composition. Carboxylated PSNPs with 100 nm were associated with more frequent detection of immunoglobulin fractions compared to that of 50 nm NPs. On the contrary, apolipoproteins were found to be in major fraction in the case of 50 nm and less frequently detected in 100 nm sized PSNPs.\textsuperscript{29} Protein interaction with various silver nanoparticles (AgNPs), via, anionic, citrate coated particles and cationic branched polyethyleneimine coated NPs with two sizes of AgNPs, 10 and 100 nm were also studied by changing ionic strength of the medium. A noticeable increase in adsorption of negatively charged proteins with cationic AgNPs was encountered. In contrast, two-fold decrease in binding of anionic proteins to both the 10 nm and 100 nm (−) AgNPs (34\% and 20\%, respectively), as compared to (+) AgNPs (71\%) was observed. It has been established that corona formation is facilitated more strongly by surface coating than that of NPs size where electrostatic interaction between NP surface and protein is dominant. A drastic increase in hydrodynamic radius upon corona formation indicated aggregation of NPs. Furthermore, change in ionic strength of the medium by the addition of cysteine and NaCl aggravates aggregation of NPs.\textsuperscript{59} The size and surface chemistry dependent protein adsorption to AuNPs with their subsequent uptake by macrophages were studied. AuNPs with different sizes, viz, 15, 30, 60, and 90 nm grafted with thiolated, methoxy-terminated PEG at densities ranging from 0 (ungrafted) to 1.25 PEG/nm\textsuperscript{2} were used for the investigation. By varying size and grafting density, NPs uptake by macrophage can be controlled. In addition, the efficiency and mechanism of NPs uptake was discussed.\textsuperscript{45} Hard corona and soft corona formations of α-synuclein on AuNPs with two different sizes, namely, 20 and 90 nm was evaluated using various spectroscopic and light scattering techniques where
AuNPs with 90 nm embrace high affinity towards binding of α-synuclein. Therefore, for same material, the size of the particle and its surface charge determines the fate of the corona composition which highly influences the nature of the proteins in corona and its biological impact.

1.2.2 Protein conformational change and their activity

The choice of surface chemistry of NPs plays predominant role in NP-protein interactions where protein conformational change is determined by the NPs surface. Studies on the interaction between HSA (human serum albumin) and QDs (quantumdots) disclosed the influence of surface functionalization of QDs by different groups DPA (D-penicillamine) and MSA (Mercaptosuccinic acid). The weak binding nature of DPA to QDs lead to less conformational change, whereas MSA causes considerable structural perturbation on interaction with HSA indicating the role of NP functionalization on conformation of protein during NP-protein interaction. The compositional study of the PC adsorbed on amine and carboxylate modified MSNs (mesoporous silica nanoparticles) as well as PEG molecules with different chain length was attempted to evaluate particle surface modification and the effect of PEGylation on particle reactivity. Unfolding behaviour of HSA upon conjugate formation with increasing negative surface charge of ligand, namely, cit-AuNPs (citrate-AuNPs) and 6MP-AuNPs (Mercaptopurine-AuNPs) were explored. Increase in chain length from CTAB (cetyltrimethylammonium bromide) to MHA (mercaptohexadecanoic acid) functionalized AuNPs displayed more reluctance toward protein adsorption. However, the amine counterpart of MHA exhibits higher protein adsorption than that of MHA. Surface chemistry of NPs served as tunable property in GOx (glucose oxidase) coverage, structure, and activity where AuNPs-PEG (neutral ligand) and AuNPs-Cys
(zwitterionic ligand) act as steric barrier for the active site with simultaneous reduction in the coverage of the GOx on the NP. The role of surface heterogeneity in tailoring of NPs was enlightened, which decide the activity of adsorbed protein molecules. BSA maintained higher esterase activity in the presence of MPA/OT (mercapto propionic acid/octanethiol) modified AuNPs than that of MPA/brOT (mercapto propionic acid/branched octanethiol), where ligands possess similar chemical composition. The effect of fibrillation of HSA in the presence of bare and functionalized MnFe$_2$O$_4$ NPs (MFN) was studied in vitro. Aminated MFN was found to inhibit fibrillation of HSA efficiently, whereas carboxylated NPs do not affect fibrillation and bare NPs reduces fibrillation to lesser extent. The electrostatic interaction between HSA molecules and MFN controls growth of fibrils by impeding the nucleation step.

1.3 Bioapplication of NP-protein interaction

The impact of NP-protein interactions can be used for various beneficial applications such as biosensors, biocatalysis and biomedicine in particular drug delivery. Nano based sensors with high sensitivity and specificity are advantageous over conventional sensors. The NP-protein interaction can be used as construct sensor, which necessitates the presence of specific interaction between NP and protein with controlled orientation of proteins over NPs surface. These interaction aids the development of sensors where NPs bound proteins recognize substrates or functionalized NPs can detect particular protein by specific interaction. RGO-AuNPs (Reduced Grapheneoxide-AuNPs) can be used for the detection of IgG (immunoglobin) using FET (Field effect transistors) device in which sensing is achieved by the specific interaction of IgG with anti-IgG immobilised over RGO-AuNPs. Detection of glycoproteins could be done by sugar-functionalized CNTs where the sugar moieties specifically interact with the
glycoprotein.\textsuperscript{69} Biocatalysis is important bioapplication of proteins where they are immobilized on NP surface, either covalently or noncovalently, resulting in enhanced protein stability. Immobilization of lipase and catalase over AuNs stabilizes enzyme by retaining 80\% of their activity even after 20 cycles where HRP (horseradish peroxidase) retains 60\%. Furthermore, the thermal stability of lipase gets enhanced upon immobilization on nanoporous gold where it retains 40\% activity even at 70\degree C.\textsuperscript{70} Immobilization of the enzyme, luciferase on SiO\textsubscript{2}NPs with subsequent delivery of NPs assisted intracellular catalysis wherein luciferase converted luciferin to oxyluciferin in cytoplasm.\textsuperscript{71} Electrochemical detection of proteins by the immobilization of nanomaterials (NMs) over electrode surface provides high sensitivity and specificity due to the specific interaction between NMs and proteins. Ultrasensitive electrochemical detection of microperoxidase-11 attached to the surface of graphene nanosheets was explored. The amplification of redox current is observed by the enhanced electroactive surface area of non-covalently functionalized graphene nanosheets.\textsuperscript{72} Further, the electrocatalytic activity of proteins can be improved by the immobilization of proteins over NP modified surface, which can be widely used for chemical and biological sensing applications.\textsuperscript{73-74} The exciting features of NP-protein interactions can be used to separate proteins, which are fundamental and essential processes in diverse biological fields. Building block-based mosaic cage silica nanotubes serves as effective anodic alumina membrane to separate proteins such as cyt c, myoglobin, hemoglobin, lysozyme, and β-lactoglobulin depending on their size by specific interaction.\textsuperscript{75} Controlled design of protein corona will enhance their therapeutic applications by providing excellent biological response. Subjugation of the biological utility of these coronas to embrace small therapeutic molecules is the current area of research. Corona stabilising AuNRs (gold nanorods) in biological environment can hold
DNA oligonucleotides and anticancer drug, Doxorubicin (DoX) whose loading capacity depends on ionic strength, assembly approach and loading concentration. Laser triggered release of molecules is accompanied by minimal leakage, though the leakage of DoX is more significant.  

1.4 Biosafety of NPs

Adequate knowledge about biosafety of NPs is necessary since the distribution, absorption, metabolism and toxicity of NPs is encoded by the NP-protein interaction. The toxicological effects of NPs and the fate of NP-protein hybrid are determined by the absorption of molecules over the surface of NPs. When the NPs are introduced into the biological system, the system recognizes molecules that are present in the outermost layer of NPs, which upon interaction with proteins gains biological identity forming protein corona. The formation of protein corona is controlled by surface chemistry and curvature of NPs as well as electrostatic attraction, hydrogen-bonding or van der Waals interactions. The NPs functionalized by hydrophobic groups are found to interact more strongly with proteins and form stable complex than that of hydrophilic and neutral counterparts, therefore, regarded as a potential safety hazard. Choice of hydrophilic groups reduces the potential risk of NPs in the application of biosystem by increasing their biocompatibility. Aminoacid functionalized AuNPs with different surface charge and hydrophobic side chains affect the binding affinity and denaturation of enzyme, Chymotrypsin. The NP-protein complex stability is enhanced by the hydrophobic interaction between hydrophobic patches of ligands and proteins. Increase in hydrophobicity of NPs surface enriches hemolytic activity compared to that of hydrophilic NPs. In contrast, corona formation over both NPs surface leads to decrease in hemolytic behavior. No hemolysis was observed within 30 min for NPs with
hydrophobic surface, which demonstrates the potential role of protein corona on the biocompatibility of NPs and therefore, allows the use of these NPs in therapeutic applications without any toxic consequences.\textsuperscript{78} Nutrient depletion, one of the cytotoxicity of NPs can be alleviated by coating NPs with excess proteins. Pretreatment of NPs with Fetal bovine serum (FBS) minimizes the depletion of nutrients like proteins and calcium in the medium thereby mitigating the cytotoxicity of NPs.\textsuperscript{79} Protein adsorption to the NP surface not only reduces the cytotoxicity of NPs but also enhances the biocompatibility and alters the dispersing/agglomerating state of NPs. Hydrophobic NMs such as CNT, fullerene and NDs (nanodots) become hydrophilic as well as water dispersible upon protein adsorption and makes them compatible to the environment.\textsuperscript{80-81} In contrast, interaction of one protein with multiple NPs induces agglomeration of NPs resulting in toxicity.\textsuperscript{82} The effect of nanomaterials (NM) and NM-PC on the fibrillation of amyloid bet (Aβ) was investigated. Though the presence of NMs (SiNPs, PSNPs, CNT) expedites the fibril formation of Aβ, their corresponding protein corona inhibits the formation of Aβ fibrils for all the NMs under consideration. Inspite of physicochemical properties (size, shape, surface composition and charge) of NMs, protein corona forms shell over NMs surface, thereby hinders the access of Aβ to the NM surface and subsequently preventing the fibril formation.\textsuperscript{83}

1.5 Techniques to portray corona formation

Formation of hard and soft corona with differential affinity or binding strength of each protein onto the specific type of NPs surface can be assessed using Fluorescence, surface Plasmon resonance (SPR), quartz crystal microbalance (QCM), and isothermal titration calorimetry (ITC).\textsuperscript{66} Poly(acrylamide) gel electrophoresis (PAGE), liquid chromatography/mass spectroscopy (LC/ MS), proteomics\textsuperscript{37, 60, 88} are used to determine
the protein identity, whereas size exclusion chromatography (SEC), differential centrifugal sedimentation (DCS) and dynamic light scattering (DLS) measure shell thickness of protein corona.\textsuperscript{30,89-90} The DLS is also used to calculate NP-protein binding stoichiometry where increase in hydrodynamic radius upon adsorption of protein to NPs surface is observed. The NMR, and MS provide information about binding sites of NP on protein.\textsuperscript{91-92} FCS can be probed to study the time evolution of protein corona formation and the shift diffusion time of the NPs can be used to determine the binding constant of protein to NPs.\textsuperscript{93} UV-visible spectroscopy coupled with multivariate curve resolution by alternating least squares (MCR-ALS) algorithm demonstrates the evolution of AgNPs–HSA conjugate by following the kinetic profiles.\textsuperscript{94} The change in protein conformation during corona formation can be studied using circular dichroism (CD) and simulations studies.\textsuperscript{64,95}

1.6 Silver Nanoparticles: Testbeds for engineered NP-protein interaction

The significant increase in biomedical applications of diverse range of NPs necessitates the need to examine NP-protein interaction. Corona formation provides biological identity to NPs which act as “Trojan Horse” that permits diffusion of NPs into the biological system. A detailed investigation on corona formation with various nanomaterials such as AuNPs, CNT, graphene, QDs, polymeric NPs, Silica NPs and oxide NPs, namely, $\text{Fe}_3\text{O}_4$, CoO, and $\text{CeO}_2$ was done.\textsuperscript{43,79,96} It was found that physicochemical properties of NMs influence protein binding irrespective of NMs.

Amongst various metal NPs, the AgNPs have gained considerable attention in diverse fields due to its unique optical, electronic and catalytic properties.\textsuperscript{97-100} These properties enabled AgNPs as potential candidate in various applications such as surface enhanced raman scattering (SERS), photonics, nanodevice fabrication, catalysis, sensors, and
biological labeling. The plasmonic effect of AgNPs coupled with electromagnetic and chemical properties makes them potential candidate as SERS substrate for the detection of analyte molecules with high sensitivity. Further, AgNPs enhances the sensitivity of SERS substrates like porous alumina membranes to monitor trace level peroxide in liquid explosives and thereby permits facile adaptation to microfluidic-based and vapor-flow Raman-detection devices.\textsuperscript{101} In situ growth of AgNPs over Si wafer demonstrates highly sensitive and specific SERS detection of DNA.\textsuperscript{102} The optical properties of AgNPs and its surface plasmon excitation are utilized in the fabrication of photonic devices. The photonic crystal effect of AgNPs enhances the emission of upconversion materials leading to the new generation of lighting devices.\textsuperscript{103} In addition to AgNPs, AgNWs (silver nanowires) also function as electron acceptor in hydrid phovoltoic devices and afford direct pathways for electrical conduction.\textsuperscript{104} AgNPs synthesized in gelatin matrix enhances the catalytic activity of TMB-H$_2$O$_2$ (tetramethyl benzidine-hydrogen peroxide) reactions which would detect Hg$^{2+}$ through label-free calorimetric assay.\textsuperscript{105} AgNPs functions as solid phase catalyst for the reduction of 4-nitrophenol when deposited on silanized magnetite beads.\textsuperscript{106} Due to their unique properties, AgNPs have also been attracted in the field of sensors, where an accurate, inexpensive, fast, and online measuring system is required. DNA mediated AgNPs and graphene quantum dots are found to be sensitive probe for the fluorescence detection of H$_2$O$_2$ and furthermore it has extended for sensing glucose by combining with glucose oxidase for the oxidation of glucose.\textsuperscript{107} An ultrasensitive electrochemical DNA assay at low attomol concentrations can be achieved by the oligonucleotide functionalized AgNPs using differential pulse volammometric (DPV) method.\textsuperscript{108} Due to the antimicrobial nature of AgNPs, it has been immensely used in the field of medicine, namely, wound healing, dental hygiene, vascular prosthesis, ventricular drainage catheters and the orthopaedics.
Furthermore, the medicinal application of AgNPs includes the use AgNPs as an anti-inflammatory, anti-platelet agents and antiviral drugs.\textsuperscript{109} Though AgNPs possess various beneficial applications, their toxicity remain as a drawback in the biomedical applications due to leakage of Ag\textsuperscript{+} ion, which is proved to be an effective toxin. It has been well established that AgNPs do not have impact toward toxicity on bacteria. However, the potential risk of AgNPs on environment as well as its antibacterial activity could be tuned by controlling Ag\textsuperscript{+} release, which can be achieved by the modification of particle size, shape, surface chemistry and aerobic condition.\textsuperscript{110} Surface chemistry of AgNPs determines their biomedical applications based on their compatibility in physiological environment than synthetic environment. The cytotoxicity and cellular uptake of AgNPs on cancer and non-cancer cell lines were evaluated based on the surface modification of AgNPs using laccose, glucose and oligonucleotides. The study indicate that the unmodified AgNPs influence the cell viability rather than that of surface modified AgNPs which would pave way for the application of modified AgNPs in cancer treatment and other therapeutic application.\textsuperscript{111} Therefore, it is necessary to focus on the suitable surface modification of AgNPs for moulding AgNPs as promising candidate in biomedical application. Furthermore, since physiological system consists of 55\% of plasma the primary interaction of AgNPs with proteins present in plasma provides knowledge on the biocompatibility of AgNPs. Although manifold reports are available for different NMs, only few studies have been focused on the interaction between AgNPs and protein along with corona formation.\textsuperscript{50, 112} Therefore, we have chosen AgNPs to study its interaction with protein and corona formation, which paves way to gain information on the biological application as well as toxicity of AgNPs.
1.7 Scope of the present work

The objective of the present thesis includes:

1. Synthesis and characterization of biocompatible AgNPs using β-Hydroxypropyl cyclodextrin (β-HPCD) as capping agent

2. Corona formation by different proteins (Hb, and Cytochrome C) over AgNPs with diverse surface characteristics (β-HPCD-AgNPs and BH₄-AgNPs) and the influence of corona formation on AgNP state, and conformational change of proteins

3. Exploiting NP-protein interaction for the determination of Bovine serum albumin using silver nanostructures by voltammetric method

1.8 References


