Chapter 7

Interaction of Gold Nanoparticle with Proteins

7.1. Introduction

The interfacing of nanoparticle with biomolecules such as protein is useful for applications ranging from nano-biotechnology (molecular diagnostics and biosensors) to medicine (therapeutic and drug delivery) to nano-electronics [1,19]. For example, the recognition properties of proteins are used in sensing and assembly of nanoparticles in to controlled structures. A better understanding of the biological effects requires knowledge of the binding properties of proteins that associate with the nanoparticle. The affinity of protein towards nanoparticles is modulated by surface properties of nanoparticles through its chemical composition, shape and size of the nanoparticles, surface functionalization, charge density etc. The conjugation of proteins with nanoparticles not only introduces biocompatible functionalities into these systems but also leads to the stabilization of the complex. The different interactions involved in nanoparticle-protein conjugate systems are van der Waals forces, electrostatic forces, hydrogen bonding etc. depending on the constituents participating in the system. The interplay of these interactions decides the formation of nanoparticle-protein conjugate structures [216-219].

There are many ways for the formation of nanoparticle-protein conjugates [216-219]. The four main approaches have been utilized so far are: (i) electrostatic adsorption, (ii) conjugation to the ligand on the nanoparticle surface, (ii) conjugation to a small cofactor molecule that the protein can recognize and bind and (iv) direct conjugation to the
nanoparticle surface. The adsorption of various proteins on gold nanoparticles has been examined under different conditions by varying protein concentration, solution temperature, pH, ionic strength etc. It has been found that the amount of protein, the composition of the protein layer, the conformational changes or rearrangement of the proteins on the particle surface vary depending on the conditions used. The nanoparticle size also strongly influences the amount of adsorbed protein and their conformational modifications. Recently, the concept of protein corona, a dynamic layer of proteins that covers the surface of nanoparticles when they come into contact with biological fluids, has been introduced. The composition of this layer depends on the affinity of the different proteins for a given nanoparticle surface. A number of techniques have been used to determine the protein-nanoparticle interactions and resultant conjugate structures [216-219].

In this chapter, we have examined the interaction of our synthesized gold nanoparticles with proteins. For this purpose, we have first optimized the synthesis to minimize any direct interaction of proteins with the block copolymer, where a stable high yield synthesis with additional reductant can be achieved for very low block copolymer concentration [220]. The interaction of these gold nanoparticles is investigated with two model proteins [lysozyme and bovine serum albumin (BSA)] at physiological conditions [221]. Lysozyme is relatively a small protein with a molecular weight of 14.4 kDa containing 129 amino acids and 4 disulfide bridges. It has an isoelectric point at pH 11. BSA has a molecular weight of 66.4 kDa and consists of 583 amino acids in a single polypeptide chain. This protein contains 17 disulfide bridges and its isoelectric point is at pH 4.7. The interaction of gold nanoparticle with proteins has been studied by UV-visible spectroscopy, zeta potential and SANS.
7.2. Experimental Procedure

7.2.1. Materials

Pluronic block copolymer P85 was obtained from BASF Corp., Mount Olive, New Jersey. The gold salt of hydrogen tetrachloroaureate(III) hydrate (HAuCl₄·3H₂O), additional reductant trisodium citrate dihydrate (Na₃C₆H₅O₇·2H₂O) and lysozyme and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, Alfa Aesar and SAF, respectively. The nanoparticles were synthesized in H₂O (Millipore) or D₂O (99.9 atom %D) as per the requirement of measuring techniques.

7.2.2. Synthesis of Gold Nanoparticles and their Interaction with Proteins

The synthesis involves the reduction of the gold salt in the presence of block copolymer in aqueous solution. Synthesis of high-yield gold nanoparticles (up to 0.2 wt% gold salt) was carried out by the addition of optimized trisodium citrate (Na₃Ct). The minimization of the use of block copolymer on high-yield synthesis was studied by systematically decreasing the block copolymer concentration by several orders of magnitude (i.e. 0.001 from 1 wt%). All the syntheses were carried out by mixing the concentrated stock solutions of the components in the sequence of block copolymer followed by Na₃Ct and gold salt, respectively at room temperature without any disturbances. The interaction of these gold nanoparticles was examined with varying concentration 1 to 5 wt% of proteins (lysozyme and BSA) at pH 7 and room temperature (30 °C).

7.2.3. Characterization of Gold Nanoparticles and Nanoparticle-Protein Conjugates

The formation of gold nanoparticles and their interaction with proteins are confirmed using UV-visible spectroscopy [19]. UV-visible measurements were carried out using 6505 Jenway
UV-visible spectrophotometer. The instrument was operated in spectrum mode with a wavelength interval 1 nm and sample holder was quartz cell of path length 1 cm.

SANS has been used to examine the structures of gold nanoparticle-protein conjugates upon their interaction. SANS measurements were carried out at SANS-I facility, Swiss Spallation Neutron Source SINQ, Paul Scherrer Institut, Switzerland [189]. The wavelength of neutron beam used was 6 Å. The experiments were performed at sample-to-detector distances of 2 and 6 m to cover a $Q$-range of 0.007 to 0.32 Å$^{-1}$. The sample solutions were kept in 2 mm thick quartz cells with Teflon stoppers. The scattered neutrons were detected using two-dimensional $96 \times 96$ cm$^2$ detector.

TEM measurements have been carried out to see the structure of gold nanoparticles during optimization of block copolymer concentration [169]. TEM has been carried out in a JEOL JEM-2100 high resolution transmission electron microscope. All HRTEM microphotographs were taken at acceleration voltage 160 kV.

Zeta potentials of gold nanoparticles and their conjugates with proteins have been determined for examining their stability. Zeta potentials have been measured with a Nanosizer Z (Malvern Instruments, Malvern, UK) by phase analysis light scattering. The light source was He-Ne laser (633 nm) operating at 4 mW. The zeta potential ($\zeta$) values are calculated from the electrophoretic mobility data using Smoluchowski approximation [222]. The experiment was carried out using a quartz cuvette (universal ‘dip’ cell) with 10 mm light pathway.

7.3. Results and Discussion

To examine the interaction of protein on gold nanoparticles requires the optimization of high yield synthesis of nanoparticles with minimum use of block copolymers [220,221]. The interaction of protein with nanoparticles has been studied by UV-visible spectroscopy and
zeta potential. Further, the resultant structures of nanoparticle-protein complexes are characterized by SANS.

7.3.1. Optimization of Synthesis

Figure 7.1. Photograph of stable gold nanoparticles at different P85 concentration for 0.2 wt% gold salt and in presence of 0.2 wt% Na$_3$Ct.

In the absence of additional reductant, the concentration of block copolymer is important to decide the yield. For example, the observance of maximum yield was changed from 0.008 to 0.016 wt% gold salt when the block copolymer concentration increased from 1 to 2 wt%. The concentration effect at such values is known to be mostly controlling the reduction and hence higher concentration of block copolymer is required to achieve higher yield. In the case of additional reductant method for higher yield, it becomes important to know the dependence on block copolymer concentration when the reduction can be controlled by the additional reductant. Figure 7.1 shows the photograph of the gold nanoparticles synthesized with varying P85 concentration (0.001 to 1 wt%) for 0.2 wt% gold salt with Na$_3$Ct. All the samples were found to be similar and equally stable. The similar SPR peak observed in UV-visible absorption spectra of the corresponding samples in Figure 7.2 clearly shows that these systems have the same yield and gold nanoparticle structures irrespective of the different block copolymer concentrations. On the other hand, the gold nanoparticle synthesis is almost fully diminished in the absence of block copolymer, suggesting that the presence of some
small amount of block copolymer must be required to enhance the synthesis. In this synthesis, the role of P85 where Na₃Ct dominates the reduction is mostly in enhancing nucleation and growth of the gold nanoparticles. The block copolymers are expected to exist on the surface of the nanoparticles providing them stability.

**Figure 7.2.** SPR peak in UV-visible absorption spectra of varying P85 concentration for 0.2 wt% HAuCl₄.3H₂O with 0.2 wt% Na₃Ct.

**Figure 7.3.** SANS data of varying P85 concentration for 0.2 wt% HAuCl₄.3H₂O with 0.2 wt% Na₃Ct in D₂O at 15 °C. The solid curves are the theoretical fits to the experimental data.
Figure 7.4. TEM micrographs of gold nanoparticles for 0.2 wt% HAuCl₄·3H₂O with 0.2 wt% Na₃Ct as prepared with varying P85 concentration (a) 0.001, (b) 0.01, (c) 0.1 and (d) 1 wt%.

The structure of nanoparticles has been characterized using SANS and TEM. Figure 7.3 shows the SANS data of gold nanoparticles synthesized from 0.2 wt% HAuCl₄·3H₂O with 0.2 wt% Na₃Ct for the varying concentration of block copolymer (0.001 to 1 wt%) at 15 °C. The measurements were carried out at a low temperature (15 °C), much less than the critical micelle temperature (CMT of 1 wt% P85 is about 29 °C) of the block copolymer P85, to avoid the scattering from the block copolymer micelles [198]. At temperature below CMT, block copolymers remain dissolved as unimers and their lower effective volume compared to when they form micelles gives rise to low scattering. All the data in Figure 7.3 have been found to have two contributions, from gold nanoparticle dominating in the lower-\(Q\) region and from block copolymer unimers at higher-\(Q\) values. The block copolymer contribution behaves as expected, to decrease with the decrease in its concentration. The data have been fitted considering gold nanoparticles as polydispersed spheres whereas the individual block copolymer by Gaussian chain. The value of radius of gyration of block copolymer is found to
be 2.3 nm and matches with that reported in literature [209,213]. The particle structure is found identical irrespective of the block copolymer concentration, having sizes about $20 \pm 2$ nm with a polydispersity of 0.4. Similar results of gold nanoparticles are also obtained by TEM as shown in Figure 7.4. The block copolymers are not seen in the TEM micrographs because of their poor electron contrast compared to that for the gold nanoparticles.

![Figure 7.5](image)

**Figure 7.5.** Integrated absorbance of SPR peaks as a function of time for varying P85 concentration with 0.01 wt% HAuCl$_4$.3H$_2$O and 0.01 wt% Na$_3$Ct.

**Figure 7.5** shows the variation of the integrated absorbance of SPR peak for different concentrations (0.001 to 1 wt%) of block copolymer while keeping the concentration of other components same. These curves can be divided in two distinct regions as (i) formation region and (ii) saturation region. The formation region is the initial period (~ 2–3 hrs) where the yield of nanoparticles increases with time. The faster formation rate is found with higher value of block copolymer concentration. The saturation region is obtained when most of the gold ions undergoing nucleation have been utilized in the nanoparticle formation. It is
interesting to note that stability and yield of nanoparticle remain same irrespective of the large decrease in block copolymer concentration.

### 7.3.2. Gold Nanoparticle Interaction with Lysozyme and BSA Proteins

Based on the optimization of different components, stable and high-yield gold nanoparticles have been synthesized even at very low block copolymer concentration [220]. This requires additional reductant (Na$_3$Ct) used in the amount approximately equal to that of gold salt to enhance the reduction of gold ions. The nucleation and growth of reduced gold ions to nanoparticles is improved in the presence of block copolymer. The additional reductant is also believed to help in stabilizing the gold nanoparticles to reduce the requirement of high block copolymer concentration. Therefore, an additional reductant block copolymer-mediated synthesis provides an ideal method for synthesizing stable and high-yield gold nanoparticles. This kind of systems can be directly used for the applications such as drug delivery where the interaction of drug with the nanoparticles is prominent. The present synthesis with low block copolymer concentration helps to minimize any direct interaction of drug with the block copolymer. The interaction of high-yield gold nanoparticles with two model proteins [lysozyme and bovine serum albumin (BSA)] has been studied [221].

**Figure 7.6.** Photograph of solutions of lysozyme protein with gold nanoparticles. The gold nanoparticles are prepared from 0.01 wt% P85 for 0.2 wt% HAuCl$_4$.3H$_2$O with 0.2 wt% Na$_3$Ct whereas lysozyme concentration is varied. The labels show the lysozyme concentrations in wt%.
The interaction of gold nanoparticles with lysozyme and BSA proteins has been found to be very different. It is observed that gold nanoparticle-lysozyme complex phase separate immediately when the two components are mixed. Figure 7.6 shows the systems of gold nanoparticles (prepared from 0.01 wt% P85 for 0.2 wt% HAuCl₄·3H₂O with 0.2 wt% Na₃Ct) with lysozyme for the concentrations 1 to 5 wt%. On the other hand, gold nanoparticle-BSA complex form a stable systems over the wide range of BSA concentration. Figure 7.7 shows the stable systems of gold nanoparticles with BSA for the concentrations 1 to 5 wt%. These results can be explained on the basis of that citrate ions are adsorbed on the gold nanoparticles make their surface negative, as a result their complex with positively charged lysozyme phase separates whereas it remains stable with similarly charged BSA (pH 7). In the case of oppositely charged nanoparticle and protein (lysozyme) the charge neutralization in the conjugate leads to the aggregation in the system. However, the site-specific adsorption of similarly charged protein (BSA) increases the overall charge in the conjugate and hence enhancing their stability. The interaction of gold nanoparticles with BSA has been examined using UV-visible spectroscopy and zeta potential.

Figure 7.7. Photograph of solutions of BSA protein with gold nanoparticles. The gold nanoparticles are prepared from 0.01 wt% P85 for 0.2 wt% HAuCl₄·3H₂O with 0.2 wt% Na₃Ct whereas BSA concentration is varied. The labels show the BSA concentrations in wt%.
The zeta potential measurement of gold nanoparticles in presence of varying BSA protein concentration is shown in Figure 7.8. For charge stabilized particles, the zeta potential is a measure of the particle stability. Typically, nanoparticles with zeta potentials greater than 25 mV or less than -25 mV have sufficient electrostatic repulsion to remain stable in solution. Gold nanoparticles prepared in aqueous solutions have a zeta potential of -39 mV and therefore are highly stable. On the other hand, it has been observed that if the gold nanoparticles prepared in aqueous solution are diluted by the buffer solution of pH 7, the gold nanoparticles become unstable. In the buffer solution the residue citrate ions continue to react with the protons in solution to form citrics, leaving gold to tend to aggregate [223]. The increase in zeta potential i.e. stability of the gold nanoparticles in the presence of BSA support to the conjugation of protein with nanoparticles.

![Figure 7.8. Zeta potential of gold nanoparticle-protein conjugates with varying BSA concentration.](image)

**Figure 7.8** shows the SPR peak in UV-visible spectra of gold nanoparticles without and with BSA. A strong SPR of the gold nanoparticles is observed whereas the presence of BSA shows a red shift in the SPR peak from 532 to 536 nm. The resonance wavelength and
bandwidth of gold nanoparticles depend on the particle size and shape, the refractive index of the surrounding medium, and the temperature. This shift is attributed to the changes in the dielectric nature surrounding the nanoparticles without and with protein conjugation [168,223].

![UV-Visible spectra of gold nanoparticles without and with BSA.](image)

**Figure 7.9.** UV-Visible spectra of gold nanoparticles without and with BSA.

### 7.3.3. Structure of Gold Nanoparticle-Protein Conjugates

SANS data from gold nanoparticles using 0.01 wt% P85 + 0.2 wt% HAuCl₄·3H₂O + 0.2 wt% Na₃Ct system, 1 wt% BSA protein and their nanoparticle-protein conjugates all prepared in D₂O are shown in **Figure 7.10**. These systems individually (gold nanoparticles, BSA and gold nanoparticle-BSA conjugate) show very different SANS data. It is also seen that SANS data of addition of contributions from gold nanoparticles and BSA is significantly different than that of the measured data of nanoparticle-protein conjugates. The build up of scattering intensity in the low-$Q$ region for the nanoparticle-protein conjugate confirms the interaction
of two components in the system. The changes in the scattering data are expected arising due to the formation of core-shell structure of adsorbed proteins on gold nanoparticle [180,181].

Figure 7.10. SANS data of gold nanoparticles, BSA and their conjugates. The gold nanoparticles in these studies are prepared using 0.01 wt% P85 + 0.2 wt% HAuCl₄·3H₂O + 0.2 wt% Na₃Ct system. The pH for BSA is kept at 7.

The SANS data of gold nanoparticles are fitted using polydisperse spheres. BSA protein macromolecules are fitted with the prolate ellipsoidal shape as is known in the literature. The adsorption of protein on the gold nanoparticles is fitted using the spherical core-shell structure consisting of adsorbed protein forming a shell around the gold nanoparticle. The fitted values of mean size and polydispersity of gold nanoparticles are 20.2 nm and 0.4, respectively. The dimensions of BSA proteins semi-major axis \(a\) and semi-minor axis \(b = c\) are found to be 7.0 and 2.2 nm, respectively. In the case of nanoparticle-protein conjugate system, proteins are adsorbed over a thickness (4.5 nm) which is about that
of the value of minor axis of the protein, suggesting adsorption of protein through the contact of major axis at the nanoparticle surface. The number of protein molecules adsorbed per nanoparticle in nanoparticle-protein complex is calculated to be 28. This number has been derived from the scattering measured on absolute scale as sum of the scattering from nanoparticle-BSA conjugates (core-shell structure) and remaining non-adsorbed BSA in the complex. It is also found that fraction of protein attached to nanoparticles is quite low about 0.05 wt%.

Figure 7.11. SANS data of gold nanoparticles, BSA and their conjugates in the gold nanoparticle contrast-matched solvent. The gold nanoparticles in these studies are prepared using 0.01 wt% P85 + 0.2 wt% HAuCl4.3H2O + 0.2 wt% Na3Ct system. The pH for BSA is kept at 7.

We have further carried out contrast variation SANS studies to confirm the protein adsorption on the nanoparticle. The gold nanoparticles are contrast matched with the mixed solvent of H2O and D2O having 25% of H2O. In this case only the scattering from protein is observed. SANS data from gold, nanoparticle, BSA protein and their conjugate under gold
nanoparticle-contrast matched condition are shown in Figure 7.11. The SANS data of gold nanoparticles show a flat pattern corresponding to incoherent background for the solvent. There is significant difference observed between the data of nanoparticle-protein conjugate and protein particularly in the low-$Q$ region. This suggests that some of the protein is part of the large structure (i.e. adsorbed shell around the nanoparticle) in the case of nanoparticle-protein system. The data fitting is found to be consistent with that in Figure 7.10. The thickness of adsorbed protein shell is obtained about 4.5 nm. The calculated number of adsorbed protein macromolecules per nanoparticle is 28 with about only 0.05 wt% protein adsorbed on the nanoparticles.

![Figure 7.12](image.png)

**Figure 7.12.** SANS data of BSA-gold nanoparticle conjugates of gold nanoparticles (0.01 wt% P85 + 0.2 wt% HAuCl₄·3H₂O + 0.2 wt% Na₃Ct) with 1 wt% BSA for two different pH values.

The mechanism of adsorption of similarly charged BSA on nanoparticles is based on the charge specificity of protein molecules. As a result, even though the net charge of the BSA protein molecule is negative it has positively charged surface patches which get
attached to the negatively charged nanoparticle surface. At pH = 7 the BSA has net negative charge whereas the gold nanoparticles also carry negative charge because of the presence of some of the citrate ions at nanoparticle surface. Figure 7.12 shows the effect of varying pH on the interaction of protein with nanoparticle. It is seen that when the pH is decreased to 5 near to the isoelectric point (4.9), the data of the nanoparticle-protein conjugate are significantly different from that at pH = 7. The scattering in the low-\( Q \) region is decreased for pH = 5 as compared to pH = 7. This indicates to the suppression of adsorbed protein for pH = 5 where the protein macromolecules are neutral. In fact, the data of individual components of nanoparticle and protein add to that the scattering of the nanoparticle-protein conjugate system. Most of the nanoparticles and protein macromolecules dispersed without any interaction at pH = 5.

Figure 7.13. SANS data of gold nanoparticles (0.01 wt% P85 + 0.2 wt% HAuCl\( _4 \cdot 3\)H\( _2 \)O + 0.2 wt% Na\( _3 \)Ct) with varying concentration of BSA. Inset shows the SANS data of corresponding pure BSA system. The pH in all the samples was 7.
Figure 7.13 shows SANS data of gold nanoparticles (0.01 wt% P85 + 0.2 wt% HAuCl₄·3H₂O + 0.2 wt% Na₃Ct) with varying concentration of BSA protein. All these nanoparticle-protein conjugate systems are found to be stable. SANS data of the conjugates show the almost similar scattering patterns in the low-\(Q\) region and difference are observed at the larger \(Q\) values. The SANS data from corresponding pure protein solutions are given in the inset of Figure 7.13. The data at higher protein concentrations show correlation peak of structure factor an indication of interacting protein in the solution. The data of nanoparticle-protein conjugate suggest that the adsorbed protein on the nanoparticles surface does not vary much with the increase in the protein concentration. The build-up of scattering in the higher \(Q\) region with increase in protein concentration is as a result of increase in the free protein (not adsorbed on the nanoparticle) concentration.

7.4. Conclusions

The optimization of high yield synthesis of gold nanoparticle for probing their interaction with proteins has been examined. The stable and high-yield gold nanoparticles have been synthesized at very low block copolymer concentration (decrease of 3 orders) to minimize any direct interaction of proteins with the block copolymer. The faster formation rate of gold nanoparticles is found with higher value of block copolymer concentration in these systems. The stability and yield of nanoparticle remain same irrespective of the large decrease in block copolymer concentration. The nanoparticle structure is also found identical irrespective of block copolymer concentration. The interaction of these gold nanoparticles with two model proteins [lysozyme and bovine serum albumin (BSA)] has been examined. It has been found that gold nanoparticles form stable solutions over a wide concentration range of BSA whereas phase separate even with small amount of lysozyme protein at physiological conditions. These results can be explained on the basis of that citrate ions are adsorbed on the gold nanoparticles make it negative, as a result their complex with positively charged lysozyme phase separates whereas it remains stable with similarly charged BSA. The
complexes of gold nanoparticles with BSA have been studied using UV-visible spectroscopy, zeta potential and SANS. The red shift in the SPR peak and the build-up of scattering intensity in the low-\(Q\) region of SANS data confirms the adsorption of protein on nanoparticles. The increase in zeta potential of the gold nanoparticles in the presence of BSA also support to the conjugation of protein with nanoparticles. The adsorption of protein on the gold nanoparticles has been modeled in SANS by a core-shell structure consisting of adsorbed protein forming a shell around the gold nanoparticle.