Protein–Nanoparticle Interaction and Its Potential Biological Implications

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Abstract

Nanoparticles in the biological environment appear in different shape and size and inside the biological milieu interact with different biomolecules. Interaction of nanoparticles with protein leads to the formation of dynamic nanoparticle–protein complex also known as nanoparticle–protein corona. However, the protein corona formed at nanoparticle interface might influence different properties of nanoparticles such as cellular uptake, accumulation, inflammation and clearance of nanoparticles. The findings from different studies on nanoparticle–protein interaction rationalized that nanoparticle interface results into conformational rearrangement of the adsorbed protein molecules, affecting the bioreactivity of the nanoparticles. The current chapter discussed on the conformational rearrangement of protein/peptide at nanoparticle interface and its biological applications. Additionally, different possible factors such as size, shape, concentration of nanoparticles and forces at nanoparticles interface affecting protein conformation are also thoroughly discussed. This chapter also highlights some important applications of nanoparticle–protein interactions like nanoparticles as possible therapeutic agents against protein amyloidosis, enhancement of antimicrobial

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propensity of peptides upon interaction with nanoparticles, use of nanoparticles as different biosensors, etc.

Keywords

Nanoparticles · Protein corona · Bioreactivity · Enzyme nanoparticles · Protein amyloidosis

8.1 Introduction

The concept of nanotechnology was first introduced by Nobel physicist Professor Richard Feynman by delivering a speech 'There's Plenty of Room at the Bottom' to the American Physical Society in December 1959. The delivered speech at the conference focused on different possibilities, i.e. if we could understand how to control even single atoms and molecules (Arakha and Jha 2018; Toumey 2009). The outcome of the speech by Professor Feynman led the scientific community to a new era of technology, known as nanotechnology. The basic aim of the technology is to formulate new molecular structures with advanced physico-chemical properties for application in various fields of science and technology. Nanoparticles are considered to be the basic fundamental units of nanotechnology, and the nanoparticles have drawn tremendous attention since they bridge the physical/chemical gap between the atomic/molecular structure and bulk (macroscopic) material (Arakha and Jha 2018). However, the definition of nanoparticles was suggested by National Nanotechnology Initiative (NNI), USA, as material with average size of 1–100 nm in at least one of the three dimensions (Kim et al. 2011). Due to unique features like high surface to volume ratio and improved percentage of grain boundaries, nanomaterials are quite different from macroscopic bulk materials (Arakha et al. 2015a, b; Fang et al. 2006). In fact, nanoparticles as the fundamental/functional units in nanotechnology possess unique physico-chemical properties, since they fall in the transition zone from the atom/molecule to macroscopic material/bulk material. The advanced physico-chemical properties of nanomaterials in comparison to bulk materials are attributed to small size, shape, surface structure, chemical composition, solubility and aggregation propensity in colloidal solution (Arakha et al. 2016, 2017; Nel et al. 2006). Hence, nanoparticles, for their enhanced properties over respective macroscopic material, are being adopted in different fields like drug delivery, diagnostic techniques, disinfectants, antimicrobial bandages, sunscreen, etc. (Meruvu et al. 2011). To complement enormous requirement by various fields of engineering and technology such as drug delivery, sunscreens, cosmetics, paints, fabrics, sporting goods and electronics, engineering of nanomaterials is growing exponentially (Miller et al. 2017; Nayak et al. 2016; Tiwari et al. 2018; Valsami-Jones and Lynch 2015; Yadav et al. 2018).

Proteins, the essential biomolecules, are synthesized on ribosome control most of biological processes inside and outside of a cell. Following the synthesis on ribosome, it folds into three dimensional structures those are further stabilized by

posttranslational modifications in eukaryotes. Hence, the three-dimensional structures of proteins determine the functions of most proteins. However, the native structure can be destabilized by perturbing the network of different interactions, like non-covalent interactions, van der Waals interactions, hydrogen bonds, hydrophobic/hydrophilic effects, electrostatic interactions, salt bridge interactions, dipole–dipole interactions, etc. (Shao et al. 2011). Unfortunately, change in local physico-chemical environment of proteins lead to the perturbation of the non-covalent interaction network, taking the conformation from folded to partially or completely unfolded conformation. Protein, in both of the cases, loses their functions resulting in degradation of proteins by proteostasis network of cell (Hipp et al. 2019). However, sometimes these confirmation goes unchecked by proteostasis network, and their accumulation results into the self-assembled pathogenic structures like amyloid fibrils (Bellotti and Chiti 2008; Jahn and Radford 2005). The self-assembly process of monomers is accompanied by many intermediate forms with cytotoxic propensity (Jha et al. 2009; Xue et al. 2009), leading to various degenerative diseases like amyloid polyneuropathy, Huntington, Parkinson, Alzheimer, type 2 diabetes, spongiform encephalopathy diseases, etc. (Bellotti and Chiti 2008). Generally, three types of degenerative diseases are reported associated with amyloid fibrils, such as (1) neurodegenerative diseases like Alzheimer's diseases where amyloid fibrils are degenerated in brain cells, (2) non-neuropathic localized amyloidoses where fibrils cause degeneration of particular kind of cell other than neuronal cells, (3) degeneration of particular kind of cell other than neuronal cells where degeneration of multiple kind of cells other than neural cells were marked (Bellotti and Chiti 2008). However, development of effective drugs against this amyloid disease is an issue for last decade. Although worldwide research is going on, however therapeutic agents to combat/hinder the amyloidoses developed till yet show insignificant effect. Since the insoluble fibrillar deposits which are irreversible in nature are the key cause for misfolded proteins, reversal of these aggregates would be an attractive strategy to formulate therapeutic agents against the protein misfolding disease (Antosova et al. 2012). In this context, first vaccine 'Doblin-based Elan Pharmaceuticals AN-1792' was developed to treat Alzheimer's disease, which was successful in case of mice but not in human. Hence, various research groups worldwide are doing research to formulate effective vaccines against amyloid diseases. Nowadays, the nanoparticles, having advanced physico-chemical properties, have attracted the attention of different researchers to inhibit amyloid fibrillation (Antosova et al. 2012; Bellova et al. 2010; Fu et al. 2009; Rocha et al. 2008).

The nanoparticles exhibit advanced physico-chemical properties in comparison to bulk materials, hence are used in drug delivery, diagnostic techniques, disinfectants, antimicrobial bandages, sunscreen, etc. (Meruvu et al. 2008; Panda et al. 2016; Sharma et al. 2018). Among the various activities of nanoparticles, interaction with protein, forming nanoparticle–protein conjugates have drawn great attention due to its direct or indirect involvement in various applications from sensing, imaging, assembly to control biological processes (Leszczynski 2010; Shang et al. 2007a). Upon conjugation with nanoparticles, protein brings biocompatibility or cytotoxic

propensity to nanoparticle. However, sometimes protein–nanoparticle conjugation leads to major/minor structural change in protein upon adsorption to nanoparticle surface (Shang et al. 2007a). The changes in protein structure on adsorption onto nanoparticle surface result in loss of the protein activity, depending upon the extent of conformational changes brought upon conjugation with nanoparticles. Additionally, conformational changes in protein on conjugation with nanoparticle may help in either enhancing or inhibiting the amyloidogenic propensity, depending upon the interaction pattern at the interface. Thus, the following headings are focused on different physical and chemical nature of nanoparticle interfaces that brings the conformational rearrangements in a protein.

8.2 Nanoparticle–Protein Interactions/Conformational Rearrangement of Protein at Nanoparticle Interface

Nanoparticles, inside the biological milieu, interact with different biomolecules, membrane, protein, DNA, etc. to further reduce its surface free energy content to attain the stability in new physico-chemical environment (Monopoli et al. 2012). Thus, as a result of the interactions, the NPs are properly dispersed in the biological environment. Nevertheless, NP interface, formed inside the biological fluids, forms attractive interactions with different biomolecular surfaces. Thus, the attractive interactions result in interface which acts against the agglomeration of nanoparticle. Among the biomolecular surfaces, the presence of protein at the interface results in complexes known as 'nanoparticle–protein corona'. As described by Monopoli et al. (2012), biological 'corona' formed due to interaction between the NP and biomolecules is considered as elements of biological identity of nanoparticles (Monopoli et al. 2012). However, different characteristics of NP like size, shape, nanoparticle composition, surface charge, surface modifications and solubility play important roles in determining the strength and kind of interaction with different biomolecules, thus the biological response and distribution (Chithrani et al. 2006; De Jong et al. 2008; Dobrovolskaia et al. 2008; McNeil 2005; Tomalia et al. 2007). Although various biomolecules are adsorbed onto the NP interface; however, the formation of protein–nanoparticle complexes has attracted the interest of various research groups as an emerging area of research (Aggarwal et al. 2009; Brown et al. 2001; Dutta et al. 2007; Goppert and Muller 2005a; Kiwada et al. 1987; Lynch and Dawson 2008; Monopoli et al. 2012; Muller and Heinemann 1989; Tyrrell et al. 1977). It is reported that generally 3700 proteins are there in plasma proteome; however, nearly 50 proteins are reported to bind with different nanoparticles (Aggarwal et al. 2009; Dobrovolskaia et al. 2009; Goppert and Muller 2005b; Kim et al. 2007). However, 'opsonins,' which are components of nanoparticle–protein corona, reported to act as a 'molecular signature', recognized by immune cells, determine the fate of the nanoparticle like kind of cell interaction, rout of internalization inside the cell, rate of clearance, distribution to different organs, etc. (Goppert and Muller 2005a; Kiwada et al. 1987; Muller and Heinemann 1989; Tyrrell et al. 1977). Interestingly, single-walled carbon nanotubes and albumin-coated silica

nanoparticles are reported to induce anti-inflammatory responses in macrophages, whereas another study reported that nanoparticle surface modified with nonionic surfactant (Pluronic F 127) to reduce the adsorption of albumin, inhibited anti-inflammatory response to the NPs (Dutta et al. 2007; Lynch and Dawson 2008). Additionally, the features of nanoparticles like rate of clearance and root of clearance from the body, organ deposition depend on nanoparticle–protein corona (Goppert and Muller 2005a; Tyrrell et al. 1977). It has been reported from various studies that all the biological responses to the NPs are possible due to surface area rather than mass (Brown et al. 2001; Donaldson et al. 2002; Donaldson et al. 1998; Muller and Heinemann 1989; Oberdorster et al. 1992). It is reported that, in some cases upon interaction with the nanoparticles, protein undergoes conformational changes resulting in loss of normal physiological function (Calzolai et al. 2010), resulting some unpredicted biological reactions including cytotoxicity (Lynch et al. 2006). Thus, the characteristic features of different nanoparticles inside the biological milieu vary depending on the physico-chemical characteristics of both nanoparticles and the biological entity.

Enzymes should retain their native structure and function for different applications in biological sciences. In this context, Asuri et al. have explored the structure, activity, and stability of different enzymes such as horseradish peroxidase, chicken egg white lysozymes, subtilisin Carlsberg by conjugating these enzymes with single-walled carbon nanotubes (SWNTs) (Asuri et al. 2007). The conjugation between different enzymes and SWNTs was covalent interaction. They have also characterized different enzymes upon conjugation with SWNTs using different biophysical techniques like circular dichroism and fluorescence spectroscopies. From extensive studies, they found that the enzymes retained their native structure and function upon attachment with SWNTs (Asuri et al. 2007). They also observed that different enzymes–SWNT conjugates are also stable in harsh chemical conditions like in guanidine hydrochloride (GdnHCl) solutions (Asuri et al. 2007). Hence, these enzyme–NP conjugates have attracted the scientists for different nanoparticle-mediated drug delivery. In another experiment, the native activity of two enzymes such as α-chymotrypsin and soybean peroxidase was observed upon adsorption onto single-walled carbon nanotubes (Wu et al. 2009). From the experiment, it was found that in case of the enzyme α-chymotrypsin, 1% of its native activity was retained, whereas in case of the enzyme soybean peroxidase, 30% of its native activity was retained (Wu et al. 2009). It is reported that the same nanoparticles help in the protein aggregation leading into amyloid fibril formation. For example, Aβ peptides are assembled to form fibrils in the presence of TiO_2 nanoparticles, since these nanoparticles accelerate nucleation process (Wu et al. 2008). De et al. have studied the refolding capacity of nanoparticles by choosing protein with positive residues on the surface (De and Rotello 2008). In their study, the protein was unfolded by thermal denaturation; hence, the hydrophobic inner cores were exposed to outside environment. The intermolecular interactions between the hydrophobic domains results in protein aggregation. They added malonic acid functionalized gold nanoparticles (AuDA) to these protein aggregates. Due to electrostatic interactions between nanoparticles having positive surface residues of

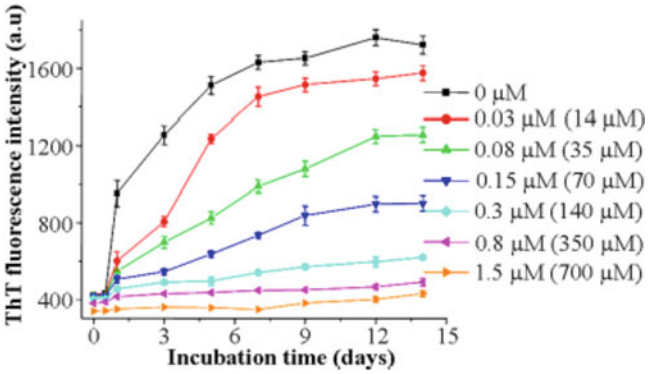

Fig. 8.1 Amyloid fibril kinetics of Aβ peptide in the absence and presence of histidine functionalized gold nanoparticles, as monitored by thioflavin T assay (Palmal et al. 2014)

proteins, nanoparticle–protein complex is formed. From zeta potential studies, they observed that high negative charge of nanoparticle–protein conjugates prevents the aggregation of the adsorbed protein (De and Rotello 2008). Shemetov et al. also observed the inhibition of Aβ-fibrillation in the presence of biocompatible nanogels (Shemetov et al. 2012).

Palmal et al. have also observed the effect of nanoparticles on Aβ-peptide fibrillation process. They incubated the peptide at different concentration of histidine-based functional groups gold nanoparticles at fibril-forming conditions. The concentration of Aβ-peptide was kept 25 μM and varied the concentration of nanoparticles from 0 to 1.5 μM. The amyloid aggregation kinetics was observed by thioflavin T (ThT) fluorescence assay (LEVINE-III H 1993). They found that the amyloid fibril formation is inhibited upon incubation with gold nanoparticle (AuNP) with histidine-based polymer coating, since ThT fluorescence intensity decreased with increase in AuNP fraction in the reaction solution (Fig. 8.1) (Palmal et al. 2014). They further observed the inhibition of amyloid fibril-like morphology when the protein is incubated with the nanoparticles using transmission electron microscope (TEM). Long amyloid fibrils were observed in the absence of nanoparticle, whereas no fibril-like morphology was visible when incubated with 0.8 μM of nanoparticles (Fig. 8.2) (Palmal et al. 2014).

8.3 Forces at NP–Protein Interface Affecting Adsorbed Protein Conformation

The different forces responsible for nanoparticle-biomolecular interaction are depicted in Fig. 8.3. Electrostatic interactions are most important forces those are charge specific. These forces attract or repel the charged protein molecules so that electrostatic double layer is formed. This charged double layer formed on nanoparticle surface creates the electrodynamic–Van der Waals interaction which may be

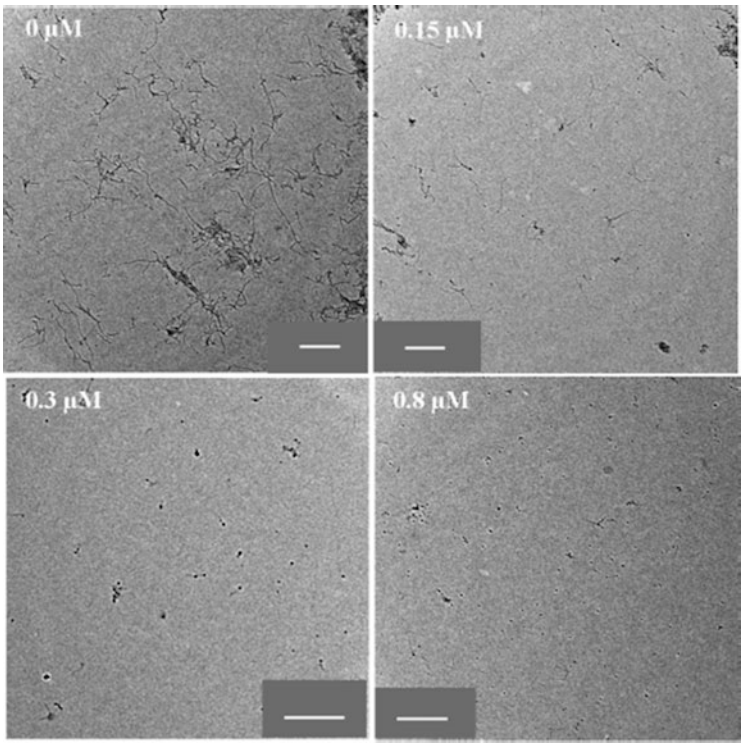

Fig. 8.2 Aβ-peptide fibrillation inhibition in the absence and presence of histidine functionalized gold nanoparticles using transmission electron microscopy (Palmal et al. 2014)

responsible for the structural and functional changes of adsorbed protein to some extent. The non-polar interactions with hydrophobic surface of protein lead to structural rearrangement of protein to a greater extent due to the exposure of inner regions of protein. This is because generally the hydrophobic domains of proteins are buried inside the protein. Though hydrophobic interactions are short range, they are responsible for the alteration of protein structure to a larger extent. All these forces described for nanoparticle–protein interaction are modulated by surface curvature of nanoparticles. It has also been studied that there is change in zeta (ζ)-potential, characteristic of charged surface with change in nanoparticles size (Shemetov et al. 2012).

It has also been studied that when the size of nanoparticle increases, there is decrease in isoelectric point of nanoparticles. It is assumed that decrease in isoelectric point is also a factor for nanoparticle interaction with biomolecules. Suttiponparnit et al. found that when particle size of TiO_2 increases from 6 to 104 nm, the isoelectric point of nanoparticle decreases from 6.0 to 3.8 (Suttiponparnit et al. 2011). These changes in isoelectric point may result in zeta potential change. Hence, the change in isoelectric point may also influence the interaction of nanoparticles with biomolecules (Shemetov et al. 2012).

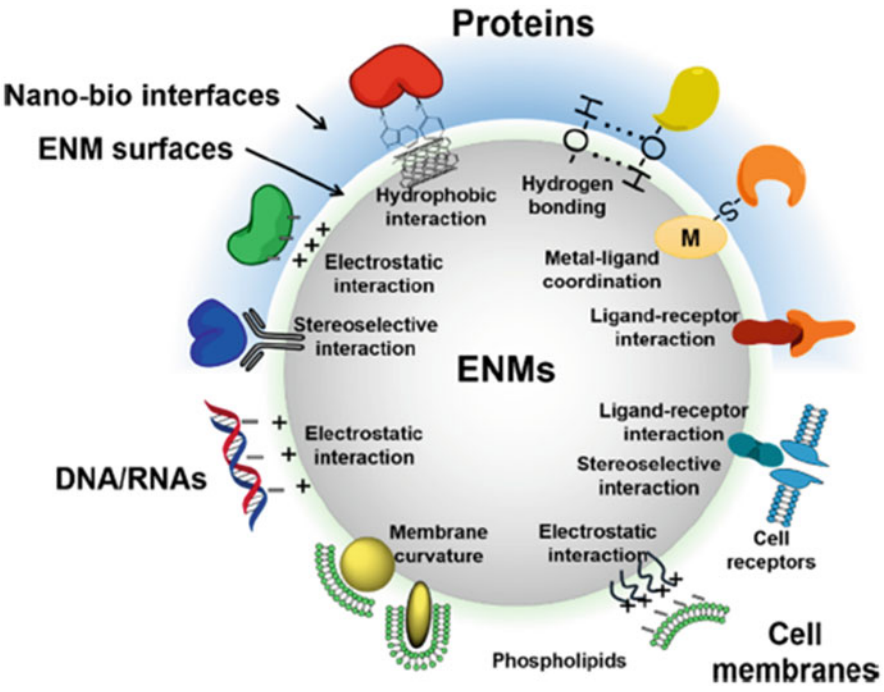

Fig. 8.3 Interactions at nanoparticle–biomolecule interface (Wang et al. 2019)

8.4 Factors Affecting Conformational Rearrangement of Protein at Nanoparticle Interface

When the biological molecules come in contact with nanoparticle interface, generally dynamic interacting components are observed, such as (1) the nanoparticle interface (the characteristics of nanoparticle interface depend on physico-chemical composition of nanoparticles), (2) the solid–liquid interface and (3) contact zone at solid–liquid interface with biological substance (Nel et al. 2009). Protein might undergo conformational rearrangement, when proteins are adsorbed onto the nanoparticle interface. However, many factors are there which are responsible for the interaction and extent of conformational changes in protein, out of which effect of size and concentrations of interfaces are discussed in succeeding headings.

8.4.1 Effects of Nanoparticle Size in Interaction with Protein

The size of nanomaterials affects the interaction pattern of protein/peptide with the nanoparticle at nanomaterial–protein interface. It has been reported that various proteins such as lysozyme, trypsin, horseradish peroxidase and catalase bind

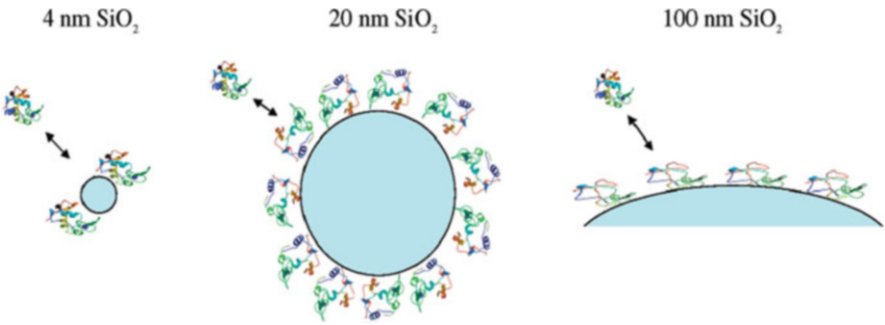

Fig. 8.4 Different sizes of silica nanoparticle affecting the interaction pattern of lysozyme with silica nanoparticle interface (Vertegel et al. 2004)

strongly to the SiO_2 nanoparticles (Vertegel et al. 2004). However, the study suggested that the partial loss of the protein structure influences significant loss of enzyme activity (Vertegel et al. 2004). As an extension of this work, Vertegel et al. have also found that the size of nanoparticle strongly influences interactions at the interface, studied by taking lysozyme and silica nanoparticle as model systems (Vertegel et al. 2004). It was found that the interaction was stronger in case of larger silica nanoparticles compared to smaller nanoparticles, leading to unfolding of lysozyme, hence resulted in insignificant lysozyme activity (Fig. 8.4) (Vertegel et al. 2004).

As shown in the figure, we can see that smaller silica nanoparticle has relatively higher surface curvature than larger silica nanoparticle. Hence, in case of protein interaction with smaller nanoparticle, the edge of the protein molecule will be at a greater distance from the NP surface, resulting in relatively weaker and non-cooperative interactions (both columbic and hydrophobic). Whereas, stronger and cooperative interactions are anticipated in case of larger nanoparticles due to the edge at closer distance. Hence, the extent of change in protein structure is relatively more significant when interacting with larger nanoparticles compared to smaller one. The loss of enzyme activity and α-helical content of lysozyme was also observed to greater extent upon its interaction with silica nanoparticles of larger size (Fei and Perrett 2009; Vertegel et al. 2004). Additionally, Shang et al. also observed similar results for silica nanoparticle upon interaction with RNaseA (Shang et al. 2007b).

8.4.2 Effect of Interface Concentration in Interaction with Protein

From extensive studies, it was reported that higher surface concentration of proteins helps in the interaction between protein molecules, because it helps in the adsorption of more proteins onto nanoparticle surface which makes a crowded environments. But, in lower concentration of protein, prominent interaction between nanoparticle and protein is observed (Fei and Perrett 2009). Wu and Narsimhan have studied the conformational changes of lysozyme upon interaction with silica nanoparticle of

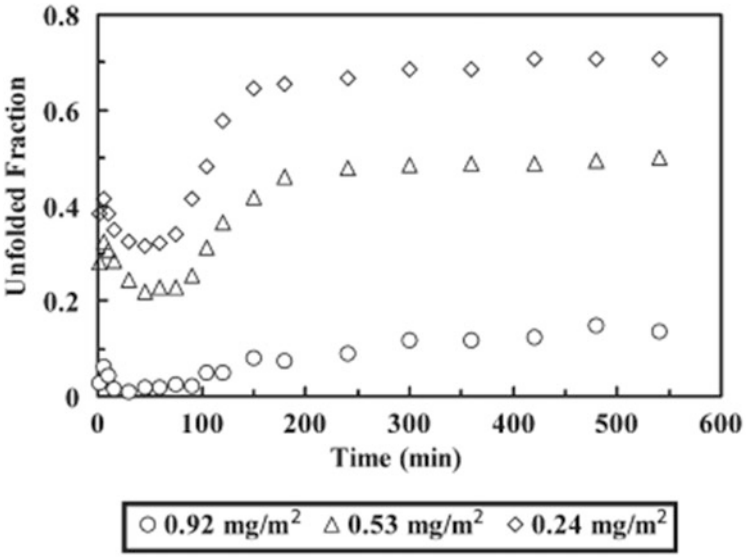

Fig. 8.5 Different surface concentration of silica nanoparticles affecting the unfolding kinetics of lysozyme at neutral pH (Wu and Narsimhan 2008)

different concentrations (Fig. 8.5) (Wu and Narsimhan 2008). At different concentrations of silica nanoparticle, the unfolded fractions of adsorbed lysozyme onto silica nanoparticle was calculated by the authors (Wu and Narsimhan 2008). From the study, it was observed that lysozyme was unfolded to a greater extent at low surface concentration in equilibrium state, which confirmed the existence of a high-energy barrier in a crowded environment (Wu and Narsimhan 2008). It is also reported that predominant interactions exists between proteins and the surface of nanoparticles at lower concentrations of protein molecules, leading to unfolding of protein, since free space is available and absence of energy barrier.

8.5 Potential Biological Implications of Nanoparticle–Protein Interactions

8.5.1 Possible Therapeutic Agents Against Protein Amyloidosis

The newly synthesized chain of amino acids fold into three-dimensional structures producing native structure. Native structure is functionally stable in local physiological conditions of protein. However, protein misfolding is a very common phenomenon of protein trafficking which occurs due to either mutations or change in local physiological chemical and physical conditions of proteins, or both. Some environmental factors, responsible for protein misfolding, are higher temperature, high or low pH, oxidative agents, elevated glucose, fatty acid level, etc. (Nelson and

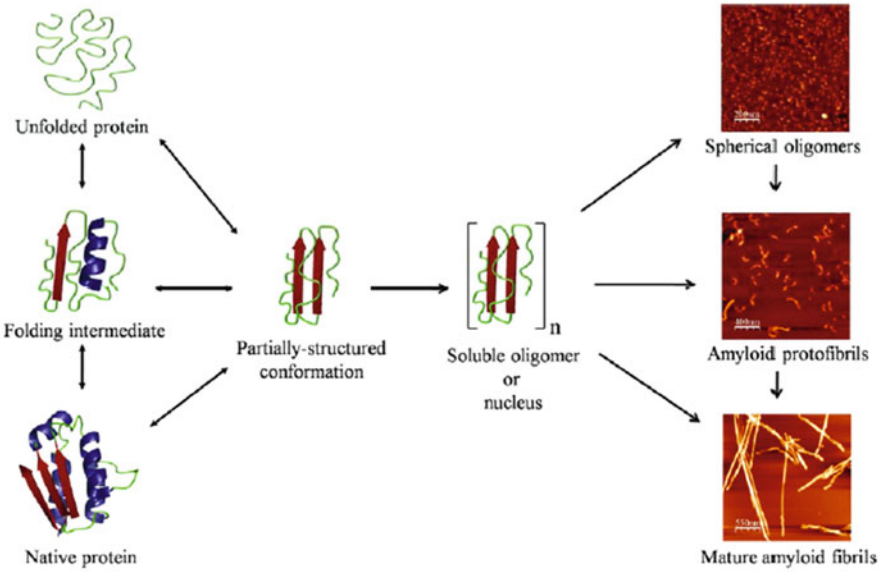

Fig. 8.6 Schematic representation of protein misfolding, aggregation. Atomic force microscopic images (right) showing amyloid fibrils (Kumar and Udgaonkar 2010)

Eisenberg 2006). After misfolding and failure of protein remodelling system, the misfolded protein kinetically or thermodynamically trapped in protein amyloid fibrillar structure (Fig. 8.6) (Kumar and Udgaonkar 2010). Protein amyloid fibrils are one specific form of protein aggregate which formed from self-assembly of misfolded proteins. These amyloid fibrils are different from other naturally occurring fibrils like collagen triple helix, keratin (Herczenik and Gebbink 2008). The most common features of amyloid fibrils are that they share a common core structure and cross β-sheet structure, and they bind fluorescent probes like Nile red, Congo red and thioflavin derivatives (Laidman et al. 2006).

When proteins are attached to planar surface, there are conformational changes in proteins. But nanoparticles are exceptional due to their high surface curvature, and less conformational changes occur to the protein. Studies have shown that some nanoparticles interact with proteins and enhance the aggregation propensity of proteins leading to the amyloid fibril formation. However, amyloid fibrils possess an alternative free energy minimum. These amyloid fibrils contain extended β-sheets aligned perpendicular to the elongation axis of fibrils. Several studies suggested that approximately 30 different proteins and peptides have been considered to be involved in the formation of amyloid fibrils inside the human body resulting in diseases (Chien et al. 2004; Chiti and Dobson 2006; Huff et al. 2003; Koo et al. 1999). Generally, in amyloid diseases, the soluble proteins self-assemble to form insoluble fibrils. It has been studied that some surfaces obtained by lipid bilayers, polysaccharides, native fibres (i.e. fibre like structure usually present in physiological condition to help cellular functions like vesicle trafficking, etc.), liquid–air,

liquid–solid, and liquid–liquid interfaces also help in either onset or prolongation of amyloid fibrillation (Knight and Miranker 2004; Myers et al. 2006; Yamaguchi et al. 2003). These studies confirmed that when proteins interact with different surfaces, physical or/and chemical adsorption of protein to the interface results in conformational rearrangement. Additionally, adsorption results in increased local concentration of the protein monomers. In case the conformational rearrangement results in exposure of hydrophobic patches or core, the increased local concentration of such monomers (conformationally compromised structure) on adsorption will result in self-assembly of the protein monomers into amyloid fibrils or other form of aggregates. In both the cases, protein monomer may not be available for usual physiological functions, hence onsets the disease.

Development of effective drugs against amyloidosis, which is strongly related to protein misfolding, has been a key issue from the last decade (Antosova et al. 2012). Worldwide research is going on to explore the novel therapeutics for the treatment of amyloidosis. The recent studies on nanoparticles have shown a novel possible approach for treating these incurable diseases (Kransnoslobodtsev et al. 2005). But unfortunately brief studies on several nanoparticles suggested that some nanoparticles like 70–200 nm copolymer particles, especially the thiol-linked nanoparticles, 16 nm hydrophilic polymer coated quantum dots, 16 nm cerium oxide nanoparticles, multiwall carbon nanotubes of 6 nm and TiO_2 nanoparticles have the potential to accelerate protein aggregation leading to fibril formation (Antosova et al. 2012). In contrast to the above findings, some nanoparticles have the potential to inhibit protein aggregation, so that these NPs can be used for the treatment of amyloidosis. Mrinmoy De et al. found that malonic acid–functionalized gold nanoparticles (AuDA) have that potential to refold the unfolded protein (De and Rotello 2008). Due to optical properties and density, gold can be easily observed in spectroscopic and microscopic techniques, and for its inert nature, it is a well-suited material for biological application (Antosova et al. 2012; Bellova et al. 2010). Bellova et al. studied the effect of magnetic (Fe_3O_4) nanoparticles on amyloid aggregation of lysozyme. They have studied this effect by thioflavin T fluorescence assay along with atomic force microscopy and found that magnetic nanoparticles interact with lysozyme amyloids in vitro. The interaction inhibited the amyloid aggregates by depolymerisation of the amyloid structure (Bellova et al. 2010). Apart from above, it has been found that fluorinated nanoparticles and hydrophobic teflon nanoparticles significantly inhibit Aβ amyloid polymerization (Rocha et al. 2008).

8.5.2 Antimicrobial Peptide Conformation at Nanoparticle Interface

Emergence of multidrug-resistant bacterial strains has become a serious threat to medical world (Arakha et al. 2016; Pal et al. 2019). Hence, different research groups are trying to develop novel antimicrobial agents against these strains. In this context, antimicrobial peptide (AMP) has attracted the interest of different research groups as

a broad spectrum of antibiotics (Pal et al. 2019). Due to the amphipathic nature of AMP, they efficiently target the membrane of microbes. However, the efficiency of these AMP has been compromised due to the emergence of these multidrug-resistant bacteria (Arakha et al. 2016; Pal et al. 2019). Hence, various research groups took the help of different nanoparticle-mediated approaches to enhance the efficacy of AMP. In this context, Arakha et al. have conjugated nisin, a widely used AMP in food industry with silver nanoparticles (AgNP). From different biophysical characterizations like UV-Vis, CD-spectroscopies and zeta potential analysis, they observed insignificant conformational rearrangement of nisin upon conjugation with silver nanoparticle (Arakha et al. 2016).

However, to evaluate the efficacy of nisin upon conjugation with AgNP, they have observed the antimicrobial activity of nisin at different ratio of AgNP-nisin conjugates (1:0.25, 1:0.5 and 1:1 w/w) against Gram-positive and Gram-negative bacteria like *Bacillus subtilis* and *Escherichia coli*, respectively, using growth kinetic analysis and colony-forming unit (CFU) measurements. From the experiments, they observed that, although nisin at nanomolar concentration shows insignificant antimicrobial activity, however upon conjugation with AgNP, the antimicrobial activity increased tenfolds higher compared to nisin (Fig. 8.7) (Arakha et al. 2016). Nisin adsorption onto AgNP enhanced the effective local concentration of nisin interaction with bacterial membrane surface, which is needed for membrane pore formation. Nisinase was sterically hindered to act upon the AgNP-adsorbed nisin. Hence, the complex was effective against the bacteria which have evolved the nisinase-based resistance against the nisin. In another study, Pal et al. have conjugated a potent AMP such as Andersonin-Y1 with AgNP and observed that the resultant conjugate exhibits enhanced antimicrobial activity by tenfolds higher against multidrug-resistant strains (Pal et al. 2019). From MD simulations, they have concluded that bacterial cell death was due to pore formation in the membrane, which is due to hydrophobic collapse mechanism. Hence, the above studies confirmed that the AMP can be a potential antimicrobial drug upon conjugation with nanoparticles against multidrug-resistant bacteria.

8.5.3 Use of Enzyme Nanoparticles as Biosensors

It has been reported that enzyme molecule can aggregate themselves to a nanoscale size and forms nanoparticle-like structure. However, the interaction playing key role here is the interaction between the enzymes forming nanoparticles. These enzyme nanoparticles are used in preparation of different nanobiosensors (Fig. 8.8). For example, nanoparticles from lipase, glycerol-3-phosphate oxidase, glycerol kinase are prepared separately, and then immobilized on an gold (Au) electrode for the preparation of triglyceride bionanosensor (Chen et al. 2017; Pundir and Aggarwal 2017). Additionally, Narwal et al. also immobilized the above three enzyme nanoparticles on pencil graphite electrode (Narwal and Pundir 2017). These enzyme nanoparticles are widely used in the construction of different biosensors for detection of molecules like triglyceride and uric acid (Chen et al. 2017). These enzyme

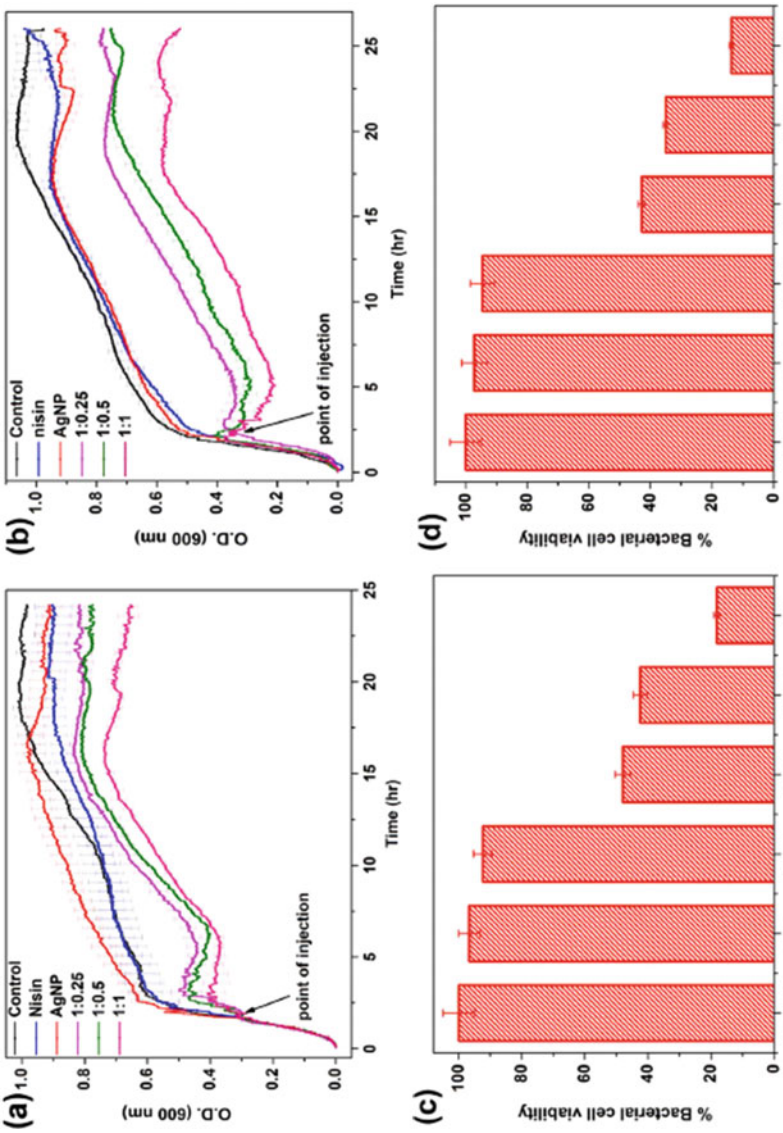

Fig. 8.7 Evaluation of antimicrobial activity of AgNP, nisin, and deferent AgNP-nisin conjugates by growth kinetics study of *B. subtilis* (**a**) and *E. coli* (**b**). Measurement of colony-forming units (CFU) for *B. subtilis* (**c**) and *E. coli* (**d**) in the presence of AgNP, nisin and deferent concentrations of AgNP-nisin conjugates (Arakha et al. 2016)

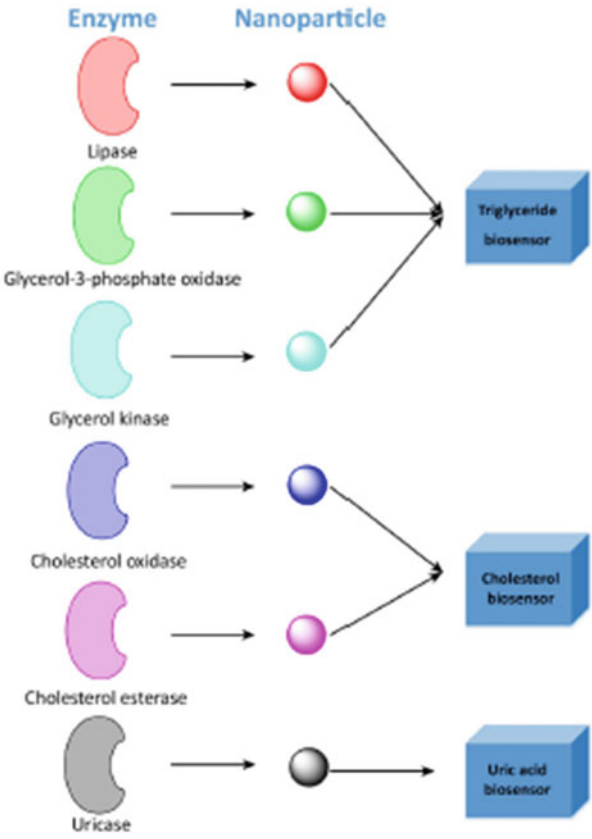

Fig. 8.8 Examples of different enzyme nanoparticles for construction of biosensors

molecules are immobilized onto different electrodes to improve the performance of biosensor (Chen et al. 2017).

8.6 Conclusion

Nanoparticle–protein interaction is a promising field for current and future research. Deep understanding of conformational rearrangement of protein upon interaction with nanoparticles can help in various ways like in treating protein misfolding diseases as well as in adopting different therapeutic approaches using nanoparticles. In this chapter, we discussed about the studies done on nanoparticle–protein interaction and the effect of nanoparticles on protein/peptide conformation. The chapter also discussed different biological applications of nanoparticle–protein interaction. Generally, most of the studies done so far were in vitro studies. Further in vivo studies are needed for better confirmation about the possible therapeutic roles of nanoparticle in protein misfolding diseases.

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