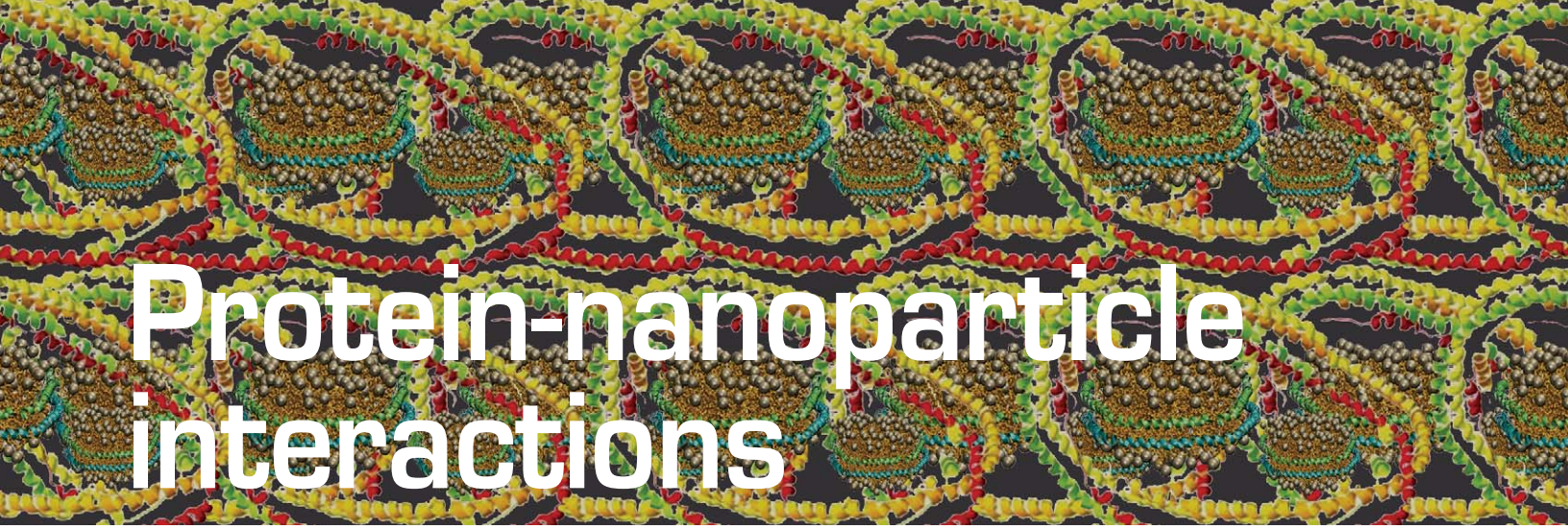

Protein-nanoparticle interactions

The key role of protein-nanoparticle interactions in nanomedicine and nanotoxicity has begun to emerge recently with the development of the idea of the nanoparticle-protein 'corona'. This dynamic layer of proteins (and other biomolecules) adsorbs to nanoparticle surfaces immediately upon contact with living systems. While within the biomaterials field the role of adsorbed molecules in cellular responses is acknowledged, there are several new issues at stake where nanoparticles are concerned. We show here that highly selective protein adsorption, added to the fact that particles can reach subcellular locations, results in significant new potential impacts for nanoparticles on protein interactions and cellular behavior.

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This article does not seek to provide a comprehensive review of all articles published on protein-nanoparticle interactions, rather it seeks to provide a snapshot of the range of activities and to highlight some of the key research directions and paradigms that are emerging and evolving in this exciting arena. An important example includes the recent discovery that nanoparticles can impact protein fibrillation processes.

Within the medical device community, it is now well accepted that material surfaces are modified by the adsorption of biomolecules such as proteins in a biological environment[1–10], and there is some consensus that cellular responses to materials in a biological medium reflect the adsorbed biomolecule layer, rather than the material itself[1,3,6]. An early study in the field of protein interactions with planar surfaces drew attention to the fact that distortion of the protein may occur upon adsorption[11]. However, the importance of the adsorbed protein layer in mediating interactions with living systems has been slower to emerge in the case of nanoparticle-protein interactions. While studies of protein adsorption to nanoparticles are beginning to appear[12–14], the importance of the detailed structure of the adsorbed protein-solution interface (the outer surface of the adsorbed protein layer taking into account any changes in protein structure) has not yet been widely appreciated in the nanotoxicology literature[1,15,16], despite the fact that this is the primary surface in contact with cells.

Of course, as one changes from a flat surface to particles, and as the particles become smaller (eventually approaching the size of the proteins themselves), the composition and organization of the associated protein will change dramatically, departing from the simple limiting case of flat surfaces. We may expect this to lead to

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quite different biological consequences. Indeed, there is the potential that highly curved surfaces (very small nanoparticles) can suppress protein adsorption to the point where it no longer occurs, an effect likely to be selective to larger proteins, offering a route to differential control of protein adsoption[17]. In addition, flat surfaces can only affect biological process via cell surface receptors such as integrins, whereas nanoparticles can enter cells and thereby access a vast range of extra biological processes.

Interestingly, rather than complicating the story, recent studies suggest that nanomaterial surfaces, which have much larger surface area than flat ones, are more amenable to studies to determine the identity and residence times of adsorbed proteins[2,18]. Indeed, direct determination of curvature effects on protein adsorption can be made, as with some material types the size of the particle can be increased until the curvature effects vanish, leaving an effectively flat surface. This offers several possibilities in terms of high-throughput or mass screening of nanoparticle-protein interactions, a concept that may have applicability as a new classification of nanoparticles based on their associated protein molecules, or 'protein corona'. We have recently introduced the concept of the 'nanoparticle-protein corona' as the evolving collection of proteins that associate with nanoparticles in biological fluids, which is, in fact, the 'biologically relevant entity' that interacts with cells[19].

Here we review some of the recent literature on nanoparticle-protein interactions in light of the fact that the biological impacts of nanoparticles are affected by the nature of the adsorbed protein layer, or the protein (biomolecule) corona. With the potential uses of nanoparticles in biological applications such as nanomedicine being well known, and the increasing importance of the emerging field of nanotoxicology, which aims to address the safety of engineered nanoparticles, the relevancy of protein-nanoparticle interactions cannot be overstated. While most of the knowledge regarding protein-nanoparticle interactions is from solution and *in vitro* studies, it is clear that future directions will require studies under competitive binding conditions such as occur *in vivo*.

The nanoparticle-protein corona

It is a (near) universal rule of materials in biology that a material is always covered by proteins immediately upon contact with a physiological environment, and we believe that this phenomenon will also be key to understanding much of the bionanoscience world[19]. We have recently argued that the effective unit of interest in the cell-nanomaterial interaction is not the nanoparticle *per se*, but the particle and its 'corona' of more or less strongly associated proteins from serum or other body fluids[1,2]. It is important to understand, though, that it is not just the composition and organization of this protein layer, but the exchange times of the proteins on the nanoparticles that is 'read' by living cells.

We conceive of the proteins associated with a particle as possessing a very wide range of affinities for the particle surface, resulting in a range of different residence times for proteins at a nanoparticle surface. In essence, we expect a huge range of equilibrium constants (one for each protein) representing the quite different (and competitive) binding mechanisms present. This means that we see the proteins associated with a particle as a 'corona', rather than a solid fixed layer (Fig. 1). The composition of the protein corona at any given time will be determined by the concentrations of the over 3700 proteins in plasma[20], and the kinetic on and off rates (or equilibrium binding constants) of each protein for the particular nanoparticle. This corona may not immediately reach equilibrium when exposed to a biological fluid. Proteins with high concentrations and high association rate constants will initially occupy the nanoparticle surface, but may also

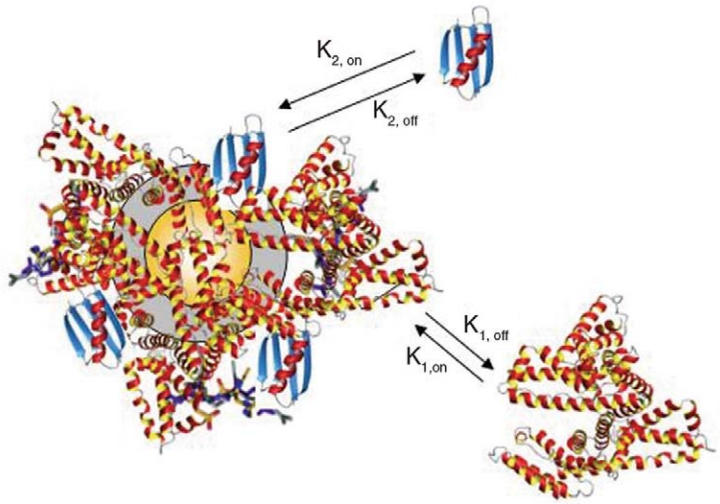

Fig. 1 Schematic representation of the protein corona on a nanoparticle illustrating the exchange processes and equilibrium constants. The exchange rates are a complex function of the affinity for the surface, curvature effects from the surface, and changes in the surrounding milieu, and much work is needed to evaluate the equilibrium constants under different conditions.

dissociate quickly to be replaced by proteins of lower concentration, slower exchange, and higher affinity[18]. Thus the protein corona is the biological identity of a nanoparticle, as it is what the cell 'sees' and interacts with. The exchange processes may also be important when particles redistribute from one compartment or organ to another, such as upon uptake into cells from the bloodstream, or upon transport from the cytosol to the nucleus.

Studies of protein adsorption bovine serum albumin (BSA), myoglobin (Mb), and cytochrome c (CytC)) onto self-assembled monolayers of mercaptoundecanoic acid (MUA) on Au nanoparticles using the quartz crystal microbalance (QCM) show that all three proteins form adsorption layers consisting of an irreversibly adsorbed fraction and a reversibly adsorbed fraction[21]. This corresponds very well with the notion of a nanoparticle-protein corona, which is proposed to have a hard corona composed of those proteins that have a strong adsorption to the nanoparticles and a long residence time and a softer corona composed of those proteins with shorter residence times and/or lower affinities.

A survey of the literature on nanoparticle-protein binding shows that the vast majority of nanoparticle types studied so far bind apolipoproteins[18]. At first sight this is a surprising result, and quite distinct from that for a flat surface. However, the fact that apolipoproteins are known to be involved in lipoprotein complexes, which themselves have sizes on the nanoscale (Fig. 2) ranging from 100 nm (chylomicron) to ~10 nm (high density lipoproteins), may mean that there are specific size-dependent interactions that drive the binding of apolipoproteins to nanoparticles. This is interesting from the point of view of nanoparticle interaction with cells, as lipoprotein complexes are involved in the general cellular processes of cholesterol metabolism[22,23]. Thus, there are multiple receptors for apolipoprotein complexes at cell surfaces that nanoparticles with surface-adsorbed apolipoproteins can potentially exploit to enter cells[24].

If we consider again the issues of nanoparticle transport and fate in animals and humans, then it is also relevant that apolipoprotein E has been found to associate to some nanoparticles[18]. This has potentially significant consequences for neurotoxicity and the development of neurotherapies, as apolipoprotein E is known to be involved in trafficking to the brain[25,26]. Thus, we hypothesize that it is the nanoparticle-protein corona (besides size and shape) that actually determines the final subcellular location of a specific nanoparticle upon interaction with a cell and, thereby, the range of disease processes that the nanoparticle can access[1]. Following on from this, we propose that, in the future, nanoparticles could be classified in terms of their biomolecule corona, which mediates their interaction with cellular machinery. This would represent a truly new paradigm in the field of nanoscale toxicology, and in the design of nanocarriers for nanomedicine.

The first reports of the direct biological influence of proteins adsorbed to nanoparticles are now emerging. Single-walled carbon nanotubes (SWNTs) and 10 nm amorphous silica coated with albumin have been shown to induce anti-inflammatory responses in macrophages, measured as inhibited induction of cyclooxygenase-2 (Cox-2) by lipopolysaccharide under serum-free conditions[27]. Blocking the adsorption of albumin by precoating the nanoparticles with a nonionic surfactant (Pluronic F127) also inhibits the anti-inflammatory properties of the nanoparticles. These observations suggest an important role for adsorbed proteins in modulating the uptake and toxicity of SWNTs and nanosized amorphous silica[27]. However, as these studies were conducted under serum-free conditions, it unclear whether the albumin would remain bound to nanoparticles under

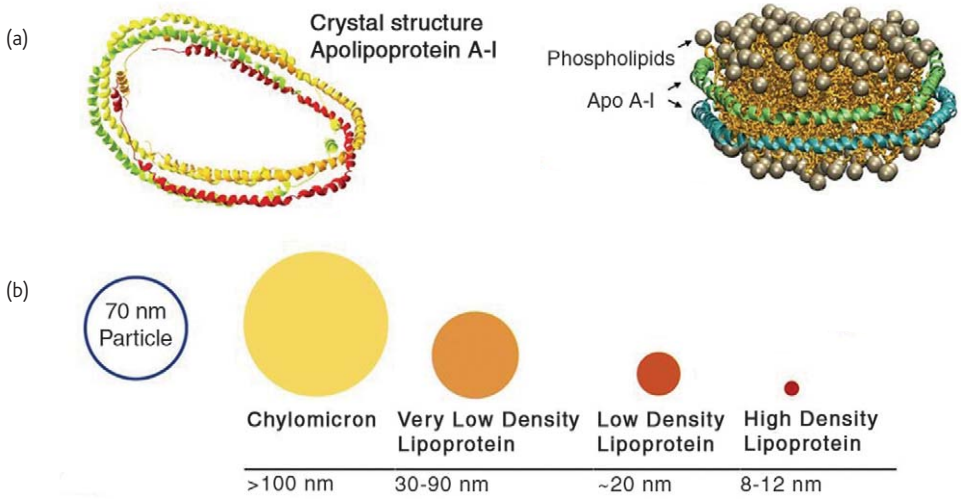

Fig. 2 (a) Crystal structure of the protein apolipoprotein A-1, and schematic of apolipoprotein A-1 in a lipoprotein complex composed of phospholipids. (Courtesy of the Theoretical and Biophysical Computational Group, University of Illinois at Urbana-Champaign.) (b) Size comparison of a 70 nm nanoparticle with lipoprotein complexes – chylomicrons, very low, low, and high density lipoproteins. These lipoproteins routinely pass into cells, and it is clear that nanoparticles of a similar size coated with apolipoprotein A-1 could be recognized as lipoproteins.

competitive binding conditions, such as occurs in plasma or in a cellular milieu.

Effects on protein conformation of binding to nanoparticles

Proteins are chains of amino acids, where the exact sequence of the amino acids determines the protein's shape, structure, and function. The principle units of protein secondary structure are α-helices and β-sheets, and the three-dimensional arrangement of these is the tertiary structure (α-helix, shown in red, and β-strand, blue, structures are illustrated in Fig. 1). The native conformation of a protein is tightly controlled by the shape complementarity of the hydrophobic residues that allow close packing of the cores[28]. Proteins are nevertheless marginally stable because the beneficial interactions that govern the native structure are counterbalanced by a large entropy loss associated with going from a large ensemble of states to a more restricted set of conformations, as well as by the repulsive electrostatic interactions present in the native state[29]. Thus, interaction with a surface can easily disrupt the native conformation and, therefore, the protein function. This has implications for the biological impact of nanoparticles.

The effect of the surface chemistry of biomaterials on the protein adsorption process has been a topic of great interest for many years, and much is known in this field[30]. Protein adsorption to various materials has been widely studied and it has been found that factors such as electrostatic interactions, hydrophobic interactions, and specific chemical interactions between the protein and the adsorbent play important roles. Selective adsorption of proteins on various synthetic adsorbents has been examined under different conditions (such as solution pH and protein concentration) and for many proteins the mechanism of selective adsorption has been attributed to electrostatic interactions[12].

More recently, many different nanoparticle and protein combinations have been studied using a range of different techniques. We have performed a detailed study of the interaction of human serum albumin (HSA) with polymeric nanoparticles of increasing hydrophobicity and size using isothermal titration calorimetry (ITC)[13], as well as studies of the interaction of complex mixtures of proteins with the same particles using techniques such as surface plasmon resonance (SPR), gel filtration, and mass spectrometry[2,18]. In the case of polymer particles (described in detail elsewhere[2]), the interaction with HSA occurs with a release of heat (a discernable enthalpy change), whereas several other proteins that we have studied (including fibrinogen, lysosyme, ovalbumin, and human carbonic anhydrase II) bind with no enthalpy change. The binding of these proteins appears to be entropy driven as a result of the release of bound water from the surface of the nanoparticle[14]. Thus, the reduction in entropy of the protein is more than compensated for by the increased entropy of the water molecules. In the case of entropy-driven binding, the interaction does not result in a conformation change of the protein. This has been confirmed by circular dichroism (CD) spectroscopy and the binding has been confirmed by SPR measurements[14].

While there are many results in the literature, we have selected a few key papers to highlight here where the nanoparticles are of particular interest commercially, e.g. quantum dots (QDs) and Au nanoparticles, which are being developed for a range of *in vivo* imaging applications, or historically, e.g. asbestos, which is the only known case to date of particle-induced toxicity. We have chosen representative papers where very pronounced nanoscale effects are noted.

The interaction between human adult hemoglobin (Hb) and bare CdS QDs has been investigated by fluorescence, synchronous fluorescence, CD, and Raman spectroscopic techniques under physiological pH 7.43. CdS QDs dramatically alter the conformation of Hb, quenching the intrinsic fluorescence of Hb and decreasing the α-helix content of the secondary structure from 72.5% to 60.8%. Raman spectra results indicate that the sulfur atoms of the cysteine residues form direct chemical bonds on the surface of the CdS QDs[15].

Geoinspired synthetic chrysotile is an asbestos reference standard, and has been used to investigate homomolecular exchange of BSA between the adsorbed and dissolved state at the interface between asbestos fibers and the biological medium. Fourier transform infrared spectroscopy (FTIR) and CD spectroscopy show that, in the solid state, BSA modifications are driven by surface interaction with the substrate[16]. Once BSA is desorbed back into solution its structure rearranges, although some of the modifications with respect to the native species are irreversible[16]. Similar effects have been observed with polystyrene nanoparticles – adsorption and subsequent desorption from polystyrene particles causes irreversible changes in the stability and secondary structure of BSA[31]. The α-helix content is reduced, while the β-turn (a region of the protein involving four consecutive residues where the polypeptide chain folds back on itself by nearly 180°)[32] fraction is increased in the exchanged molecules. The irreversible surface-induced conformational change may be related to the aggregation of BSA molecules after exposure to a hydrophobic surface[31].

The effect of the curvature (angle) between the crystal faces of peptide-protected Au nanoparticles (which are polyhedral species) on the secondary structure of peptides is an unexplored but potentially critical issue. A recent structural investigation of a peptide containing 16 amino acids in both two- and three-dimensional self-assembled monolayers (SAMs) with increasing core diameters, and therefore decreasing curvature between the crystallographic faces, shows that the degree of surface curvature has a profound effect on the secondary structure of the peptide. In addition, a three-dimensional monolayer (on Au nanoparticles) does not always resemble the two-dimensional monolayer on a Au surface[33].

Functionalization of nanoparticle surfaces with peptides is increasingly being used to control the interaction of nanoparticles with proteins[34,35]. Investigation of the effect of Au nanoparticles with

positive, negative, and neutral ligands on attached CytC structure reveals that the protein retains its structure with neutral ligands but denatures in the presence of charged species[34]. Similar studies of the interaction of Au nanoparticles functionalized with L/D-leucine and/or L/D-phenylalanine residues with alpha-chymotrypsin (ChT) and CytC show that the chirality of the functionalized nanoparticle end-groups substantially affects the resultant complex stability, with up to 20-fold differences between particles of identical hydrophobicity. This demonstrates that structural information from the ligands can be used to control protein recognition[35].

Methods to evaluate protein binding to nanoparticles

Many methods are commonly used to study nanoparticle-protein interactions, several of which have already been mentioned in the sections above, such as FTIR, CD spectroscopy, ITC, SPR, mass spectrometry, and fluorescence spectroscopy[2,13,15,16,18,31].

We have recently reported the use of size-exclusion chromatography to study nanoparticle-protein interactions, with specific emphasis on the determination of the residence times of proteins on nanoparticles in order to identify those proteins that may be relevant on the biological timescale[2]. This method is based on the fact that nanoparticles are too big to enter the pores of the size-exclusion matrix, and thus pass through the column in the void volume, whereas proteins are small enough to enter the pores, and the time they spend inside the pores depends on their molecular weight. Thus, each protein elutes with a characteristic volume. However, in the presence of nanoparticles, the elution volume of proteins is shifted toward earlier elution times depending on the duration of the interaction between the protein and the nanoparticle – that is, the protein hitches a ride through the column on the nanoparticle, and thus the nanoparticle-associated proteins are separated from the nonassociated proteins (Fig. 3). Using this technique, we have been able to identify those proteins that bind, as well as their association times[2].

The techniques reported here are a mixture of the easily accessible (fluorescence and CD) and the highly specialized (SPR and mass spectrometry). This distinction is an important one in terms of the mass screening of protein-nanoparticle interactions to characterize nanoparticles in terms of their protein corona. In order for this to become feasible for the screening/characterization of the 30 000 nanoparticles that are claimed to be in the industrial pipeline worldwide, the experimental techniques need to be robust, easily accessible, and high throughput (where possible). We highlight here another approach that could potentially contribute to large-scale screening of nanoparticle-protein interactions, namely zeta potential.

The interaction of negatively charged BSA and positively charged lysozyme with a range of metal oxide particles (alumina, silica, titania, and zirconia particles with diameters 73–271 nm) has been studied by zeta potential[36]. The adsorbed proteins change the zeta potentials and the isoelectric points (IEP) of the oxide particles. The amount of adsorbed protein on the alumina, silica, and titania (but not on the zirconia) particle surfaces correlate with the zeta potential. For the slightly less hydrophilic zirconia particles, significant amounts of protein adsorption are observed even under repulsive electrostatic conditions, perhaps on account of the hydrophobic effect playing a more important role for zirconia than the electrostatic interactions[36]. While this data alone does not provide much insight into the effects of the interaction on protein conformation, it may be possible to correlate the zeta potential of nanoparticle-protein complexes with the nature of

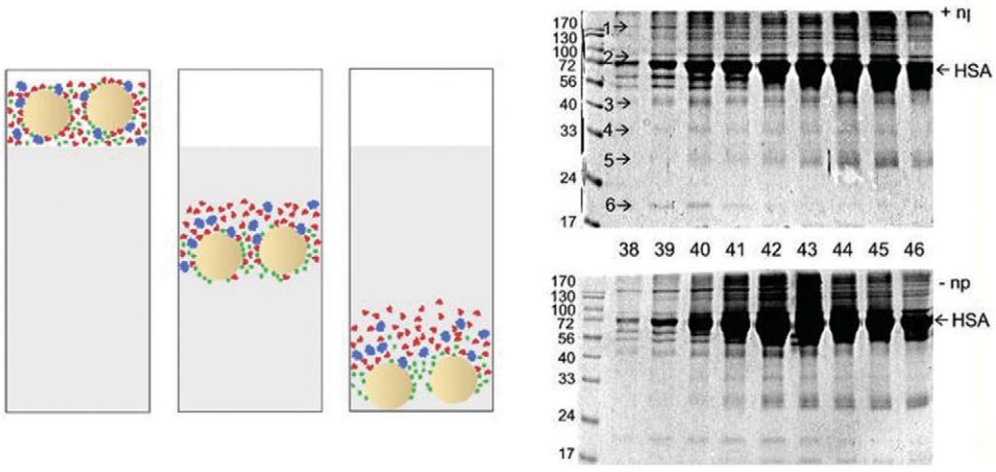

Fig. 3 Size-exclusion chromatography study of nanoparticle-protein interactions. The elution time of proteins is shifted depending on their affinity for the nanoparticle surface, the longer the protein is associated with the nanoparticle the earlier the protein elutes from the column[2]. Proteins that have sufficiently long residence times elute in the void volume with the nanoparticles. It is clear that each fraction collected from the size-exclusion column contains many different proteins, which can be further separated by gel electrophoresis using denaturing acrylamide gels as shown on the right. The different gel bands can be cut out and the proteins identified by mass spectrometry. (Reproduced with permission from[2]. © 2007 National Academy of Sciences.)

the principle proteins adsorbed and, in the longer term, to correlate this to nanoparticle uptake by cells.

Increased protein stability/activity upon binding to nanoparticles

While, in general, the loss of secondary structure and consequent changes in the activity of proteins upon binding to nanoparticles can be seen as a drawback or a potential source of nanoparticle toxicity, there is a potential positive outcome too. Promising uses of nanoparticles include increasing protein stability toward enzyme degradation and increasing the activity of enzymes via immobilization at surfaces. Enzymes such as Candida rugosa lipase (CRL) and Pseudomonas cepacia lipase (PCL) have been adsorbed to nanostructured polystyrene (PS) and polymethylmethacrylate (PMMA) by simple addition of the lipase solution to the polymeric nanoparticles under protein-friendly conditions (pH 7.6)[37]. Adsorption leads to improved performance in terms of activity and selectivity with respect to that shown by lipases adsorbed on the same non-nanostructured carriers, as well as increased enantioselectivity and pH and thermal stability[37].

The highly curved surface of C_{60} fullerenes have also been shown to enhance enzyme stability in strongly denaturing environments to a greater extent than flat supports. The half life of a model enzyme, soybean peroxidase, adsorbed onto fullerenes at 95°C is ~2.5-fold higher than that of the enzyme adsorbed onto graphite flakes and ~13-fold higher than that of the native enzyme[38]. Similar observations have been found with other nanoscale supports including silica and Au nanoparticles. The ability to enhance protein stability by interfacing them with nanomaterials may impact numerous fields ranging from the design of diagnostics, sensors, and nanocomposites to drug delivery[38].

Potential role of nanoparticles in protein fibrillation

Amyloidogenic proteins are a group of proteins that aggregate under certain conditions to form highly insoluble structures (fibrils), which precipitate to form plaques. These aggregates are involved in a range of serious and irreversibly progressive pathological conditions (protein-misfolding diseases), such as Alzheimer's disease, Parkinson's disease, and dialysis-related amyloidosis. At least 20 such diseases are known.

We have reported recently that a range of different nanoparticles, including polymer particles, cerium oxide, carbon nanotubes, and poly(ethylene glycol) (PEG)-coated QDs, enhance the rate of fibrillation of the amyloidogenic protein β-2-microglobulin under conditions where the protein is in a slightly molten, globular state at pH 2.5[39]. As the fibrils imaged by transmission electron microscopy (TEM) do not appear to grow out of the nanoparticles (Fig. 4), we have suggested a mechanism based on the locally increased concentration of the protein in the vicinity of the nanoparticle surface. This, we believe, increases the probability of the formation of a critical oligomer that, once formed, returns to the solution phase. Multiple layers of protein bound to the particles and interaction with the particles does not appear to have much effect on the protein conformation as determined by fluorescence of the tryptophan residues, with slight effects observed for the innermost protein layer, and no observed effects for subsequent layers[39].

A more recent report from Bellezza *et al.*[40] suggests that the interaction of Mb with phosphate-grafted zirconia nanoparticles induces significant rearrangements in the Mb structure, particularly loss of the secondary structure (α-helices). The amount of bound Mb implies a monolayer of adsorbed molecules. Atomic force microscopy (AFM) measurements indicate that the interaction also affects

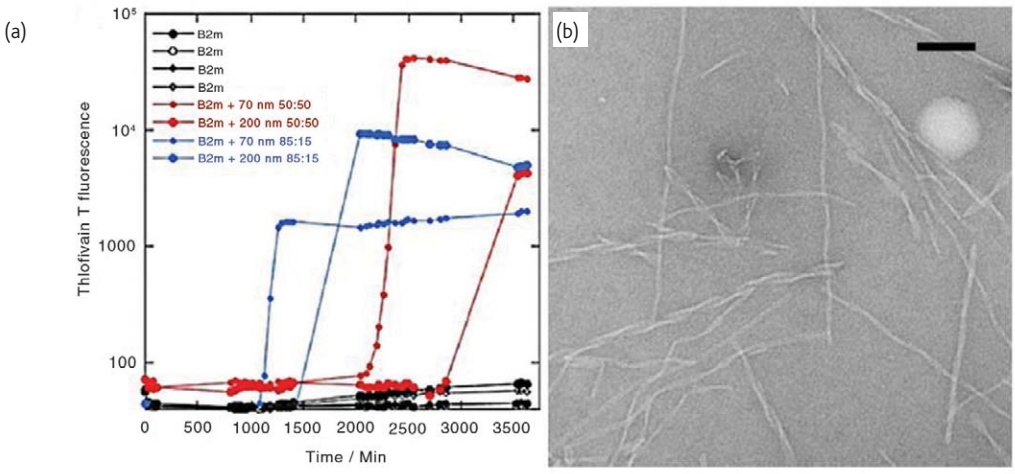

Fig. 4 Nanoparticles have been shown to increase the rate of fibrillation of amyloidogenic proteins using assays based on the binding of thioflavin-T to protein fibrils[39]. The presence of 70 nm and 200 nm polymeric particles results in a reduced fibrillation time for β-2-microglobulin (B2m), the protein involved in dialysis-related amyloidosis. (a) Thioflavin-T assays in the absence (black) and presence of nanoparticles of different size and composition. As the thioflavin-T only fluoresces when it is bound to fibrils, the onset of fluorescence correlates with the onset of fibrillation. (b) TEM of the protein fibrils in the presence of nanoparticles showing that the fibrils do not grow out from the nanoparticles. Scale bar: 100 nm. (Reproduced with permission from[39]. © 2007 National Academy of Sciences.)

the morphology of the bound protein, inducing the nucleation of prefibrillar-like aggregates at pH 4.7, whose appearance and height (2–4 nm) are consistent with the prefibrillar-like assemblies previously seen for Mb[40]. It is well known that Mb can nucleate fibrillar structures only under destabilizing conditions, where it is has at least a partially unfolded conformation[41]. In this case, the authors state that the prefibrillar-like aggregates are always observed next to the ZrO_2–P nanoparticles, suggesting that the prefibrillar-like structures develop from the bound protein.

Other nanoscale structures have also been recently reported to induce protein fibrillation: dendrimers (synthetic, symmetrically branched polymers that can be manufactured to a high degree of definition and, therefore, present themselves as monodisperse entities) interact with and perturb polypeptide conformations, with particular efficiency toward amyloid structures. Various cationic dendrimers have been shown to be potently disaggregative toward prion aggregates, and to be able to remove prion molecules in the infectious state (PrPSc) effectively from both PrPSc-infected neuroblastoma cells and from PrPSc-containing brain homogenates[42]. However, the same dendrimers have variable effects on the stability of different proteins, suggesting that they do not act as generic denaturants, but rather exert their effects via specific interactions with individual parts of each protein.

Studies in animals have shown that C_{60} hydrated fullerene may have anti-amyloidogenic capacity resulting from inhibition of the fibrillation of amyloid-beta 25–35 peptide[43]. A single intracerebroventricular injection of a C_{60} hydrated fullerene at a dose of 7.2 nmol/ventricle significantly improves the performance of a cognitive task in control rats. TEM studies have confirmed that C_{60} hydrated fullerene inhibits the fibrillation of amyloid-beta 25–35 peptide. This suggests a potential role for nanoparticles in the development of therapies against amyloidogenic diseases. There is currently no cure or reliable diagnosis until postmortem in many cases (e.g. Alzheimer's disease), so advances such as these are very exciting.

Use of nanoparticles to probe protein-protein interactions

In order to fulfill their biological roles, proteins must interact with other molecules. For instance, enzymes, receptors, and transcription factors have to bind their substrates, ligands, and target DNA elements, respectively, to execute their function. Thus, small changes in protein conformation, as could be induced by interaction with the surface of an engineered nanoparticle, can have significant impacts on a protein's function and on its interaction with other proteins (protein-protein interactions). In addition to identifying the proteins directly involved in a nanoparticle's corona, it is also important to understand the effect of incorporating a protein into a nanoparticle corona on the protein-protein interactions, in order to ascertain the biological impacts induced by nanoparticles.

Nanoparticles and nanostructured surfaces offer a new route to study protein interactions, both protein-ligand and protein-protein. For example, we have studied the binding of HSA to a series of polymeric nanoparticles of increasing hydrophobicity in the presence and absence of oleic acid[13], which is one of the key ligands that HSA binds during its normal functioning[44]. Very different interaction patterns are observed with and without oleic acid – HSA in complex with oleic acid gives an endothermic signal and lower stoichiometry compared with the exothermic signal from apo-HSA (in the absence of oleic acid), as shown in Fig. 5. A recent study using semiconductor nanocrystals (QDs of average diameter less than 2 nm) directly conjugated to HSA

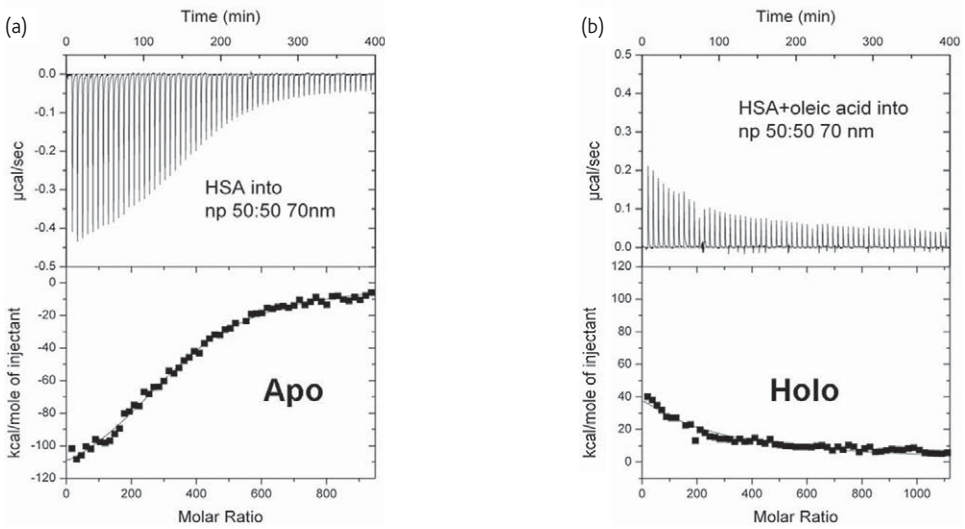

Fig. 5 ITC has been used to study nanoparticle-protein and nanoparticle-protein-ligand interactions[13]. The graphs show the binding of HSA in the absence (a) and presence (b) of oleic acid (one of the major ligands that binds to HSA) to 70 nm hydrophobic nanoparticles. The binding isotherms also give information about the number of molecules bound and the energetics of binding. In these studies, 1 mg/ml of HSA was titrated into 10 mg/ml particles. (Reproduced with permission from[13]. © 2007 American Chemical Society.)

has shown that these conjugates can be used as fluorescent biological labels. Using measurements of fluorescence resonance energy transfer (FRET) from the amino acid tryptophan (Trp214) to the QD, it is possible to follow the local and global changes in the HSA structure during thermal unfolding and refolding processes[45].

Recently, a dual-functionalized nanoparticle has been described that combines histidine-tagged protein purification with site-specific fluorophore labeling[46]. The particles are tetramethylrhodamine-doped nickel chelate silica nanoparticles surface modified with nitrilotriacetic acid. When exposed to a bacterial lysate containing the estrogen receptor alpha ligand binding domain (ERalpha) as a minor component, these beads show very high specificity binding, enabling protein purification in one step. The particles also exhibit good activity for ligand binding and ligand-induced binding to co-activators in solution FRET experiments and protein microarray fluorometric and FRET assays[46].

From the range of studies presented here, it is clear that research into nanoparticle-protein interactions is a dynamic area but there is much still to be done. There also appears to be considerable contradiction in terms of the findings, with for example, increased protein stability upon binding reported for some protein-nanoparticle pairs and protein destabilization reported for other protein-nanoparticle pairs. Also, the effects of nanoparticles on protein fibrillation seem to be nanoparticle-protein pair specific. Thus, we are some way from general paradigms as yet, although the nanoparticle-protein corona as the biological identity of nanoparticles does appear to be an overarching effect, with the potential to unify various findings into a general approach to characterizing nanoparticle-protein interactions.

Conclusion

Our core hypothesis is that, rather than the simple nanoparticle itself, it is the dynamical corona of associated biomolecules that defines the biological identity of the nanoparticle. It is therefore this corona that might lead to a more clear classification system of nanosafety, and it is also this corona that could be used to engineer nanomedical outcomes. We therefore believe that very considerable efforts will increasingly be directed at this challenge by the scientific community.

We also note the surprising feature that nanoparticles are sometimes able to induce dramatic effects on protein interactions, such as in the case of protein fibrillation. This observation, when combined with the potential of nanoparticles to be transported to the brain, implies the need to study this whole arena in more depth in the future.

Scientists in the 20th century developed surface physical chemistry and related fields to a high point of achievement. It is an interesting observation that once more, albeit in quite a different context, high surface area adsorption of biomolecules has become a key arena for the development of bionanointeractions. nt

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