Molecular Interaction of Proteins and Peptides with Nanoparticles

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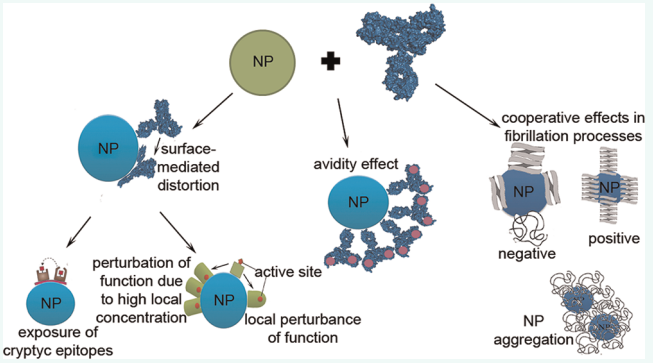
ABSTRACT



The interaction of proteins in living cells is one of the key processes in the maintenance of their homeostasis. Introduction of additional agents into the chain of these interactions may influence homeostatic processes. Recent advances in nanotechnologies have led to a wide use of nanoparticles (NPs) in industrial and biomedical applications. NPs are small enough to enter almost all compartments of the body, including cells and organelles, and to complicate the pattern of protein interactions. In some cases, interaction of nanoscale objects with proteins leads to hazardous consequences, such as abnormal conformational changes leading to exposure of cryptic peptide epitopes or the appearance of abnormal functions caused by structural modifications. In addition, the high local protein concentration resulting from protein adsorption on NPs may provoke avidity effects arising from close spatial repetition of the same protein. Finally, the interaction of NPs with proteins is known to induce cooperative effects, such as promotion or inhibition of protein fibrillation or self-assembling of NPs on macromolecules serving as a template. It is obvious that better understanding of the molecular mechanisms of nano–bio interactions is crucial for further advances in all nanotechnological applications. This review summarizes recent progress in understanding the molecular mechanisms of the interactions between proteins or peptides and NPs in order to predict the structural, functional, and/or nanotoxic consequences of these interactions.

KEYWORDS: protein–nanoparticle interaction · proteome · protein aggregation · self-assembly · surface forces · quantum dots · colloidal nanocrystalls · amyloidosis · protein structure

The rapid development of nanotechnology in the past decades offers wide prospects in using micro- and nanoscale materials in different areas of industry, technology, and medicine. This is why understanding the mechanisms of interactions between nanoparticles (NPs) and living matter is crucial for safe implementation of nanotechnologies in various fields. Therefore, we need to understand how NPs enter the body, tissues, and cells, where they go when they get there, and what are the consequences of them being there. Furthermore, if we want to fully understand the biological impact of NPs, we should address all the complicated molecular aspects of nano–bio interactions.

Routes of Nanoparticle Entrance into the Body and Potential Hazards of the Interaction of Nanoscale Objects with Living Matter. There are a lot of examples of using NPs for drug delivery[1−4] and imaging, as well as labels or sensing probes.[5−7] Before their arrival to the final intrabody or intracellular destination, NPs should penetrate into the body and move or be delivered to the target compartment. The human body has several semiopen interfaces for direct substance exchange with the environment, including the respiratory system, gastrointestinal tract, and skin (Figure 1).[8] At these interfaces, NPs encounter biological barriers and may undergo different processes, such as peptide or protein binding, phagocytosis, deposition, clearance, and translocation. In most cases, the next step of NP delivery to the target location is their entry into the bloodstream, where NPs may interact with new targets and interfaces—blood components and vascular endothelium.[9−11]

The respiratory system is one of the most common ways of the NP entry into the body (Figure 1). This pathway is successfully used by NPs less than 2.5 μm in size, whereas larger particles are removed from the respiratory tract by the mucociliary escalator.[8] Deposition of inhaled ultrafine particles (with the hydrodynamic diameter <100 nm) mainly takes place in the alveolar region. NPs come into contact with the fluid surfactant layer lining the alveoli, where, depending on the nature of the nanoparticles, reactive oxygen species may be generated in the strongly oxidizing environment.[12] Then, NPs are eliminated from the alveolar region through several major routes. The first route

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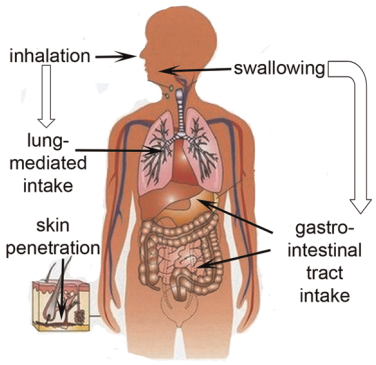

Figure 1. The main pathways of nanoparticle penetration into the human body. Inhalation of particles leads to their intake through the alveolar system to the blood or lymphatic system. Oral administration leads to intake through the gastrointestinal tract. Skin penetration is followed by blood intake or skin distribution of the particles.

is the mucociliary escalator transport along the tracheobronchial tree, the second one is NP translocation into the lymphatic system *via* their uptake by macrophages, and the third one is NP dissolution followed by the transfer of the products into the blood through interaction with squamous epithelial cells of the alveoli.[13] Another way is the interaction of NPs with alveolar macrophages, which are also involved in the elimination of inhaled NPs. Alveolar macrophages are the key component of the deposition/clearance mechanisms. These cells are recruited to eliminate deposited NPs, their phagocytic capacity and responsiveness to the phagocytic stimulus determining the fate of the NPs.[14] Deposited NPs, partly through interaction with lung epithelial cells, can induce rapid recruitment of macrophages.[15,16] In addition, it has been shown that inhaled ultrafine particles, such as carbon and TiO_2, impair the phagocytic function of alveolar macrophages.[16,17] Phagocytosis of NPs leads to activation of macrophages and release of chemokines, cytokines, reactive oxygen species (ROSs), and other mediators, which may result in sustained inflammation.[18,19] The high inflammogenicity is assumed to be determined by the small sizes and large surface area of ultrafine particles, which promotes free radical generation.[20] Inhalation experiments have shown that ultrafine silver NPs are taken up by alveolar macrophages; aggregated silver particles are retained in these cells for at least 7 days.[21] At high concentrations of NPs, their interaction with macrophages may cause inflammatory conditions in the lung, such as asthma, chronic obstructive pulmonary diseases, or respiratory infection.[22] Moderate concentration of NPs may induce autophagy[23,24] or apoptosis[24,25] of lung cancer cells, which may be considered a positive consequence of NP penetration, although higher concentration of NPs may lead to their accumulation and subsequent damage to healthy cells, too.[26]

Another route of the NP entry into the body is the skin (Figure 1).[27,28] The skin accounts for more than

VOCABULARY: protein aggregation – as a result of mutation-related misfolding, posttranslational modifications or changes in local conditions (*e.g.*, thermal, osmotic, and oxidative stresses; UV irradiation; and critical pH changes), the secondary and tertiary structures of protein molecules may undergo transformation that result in amorphous or highly structured fibril-like aggregates; **ζ-potential** – an electrochemical parameter defined as the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle; **avidity effect** – the sum total of the interactions between molecules at multiple binding sites. This effect is distinct from affinity, which is the strength of interaction of one molecule with another at a single binding site; **amyloidosis** – a variety of conditions whereby the "bad proteins" referred to as amyloid proteins are produced in the body. Misfolded amyloid proteins are abnormally deposited in organs and/or tissues and cause strong pathological changes. Amyloidosis may be either primary (with no known cause), secondary (caused by another disease, including some types of cancer), or hereditary; **cooperative effect of interaction** – two interaction events are said to be cooperative if one interaction event enhances (a positive cooperative effect) or disturbs (a negative cooperative effect) the other interaction event;

10% of the body mass and plays the important role of a barrier against the external environment. Despite the strong barrier function, there is evidence that various types of NPs can go through it. For instance, micrometer-sized TiO_2 particles, a common ingredient of sun-protection cosmetics, easily penetrate through the epidermis and dermis;[29] semiconductor quantum dots (QDs) with various physicochemical properties could penetrate through an intact stratum corneum barrier and accumulate within the epidermal and dermal layers;[30] and fullerene-based peptides have also been shown to penetrate through intact skin.[31] Although intact skin is pervious to NPs, skin wounds may provide an easier penetration. This pathway may ensure a more rapid penetration of NPs into dermal capillaries and their distribution in the body. This is also a presumed pathway of NP penetration into the lymphatic system, which, depending on the nature of NPs, may lead to additional inflammation processes. For example, accumulation of silver NPs by mast cells may cause argyria, irreversible pigmentation due to deposition of silver NPs.[32,33] Numerous mast cells containing silver particles in their cytoplasm and exhibiting piecemeal degranulation have been found around silver particles in the dermis of a patient with localized argyria.[32] The pathway of mast cell degranulation in metal-induced allergic and autoimmune reaction was analyzed in detail. It has been shown that silver activates mast cells *in vitro* by inducing production of ROS and oxidation of thiols, which is crucial for metal-induced Ca^{2+} influx.[34] Moreover, the inflammatory and toxicological effects

of nanosilver on macrophage strongly depend on the particle size. A high reactivity of 4-nm silver nanoparticles and their easy transportability into immune cells as compared to the 20-nm and 70-nm nanoparticles seems tobe the substantial difference in their inflammatory potential.[35] Epidermal keratinocytes (HEK) have also been shown to be capable of phagocytosing multiwalled carbon nanotubes (MWCNTs) and setting off inflammatory responses through realizing interleukin 8.[36] Mechanical irritation and interference with dermal resident microflora by nanosilver-based fibers might also pose potential problems. In addition, some other types of NPs, for example, sulfonated multiwalled carbon nanotubes (S/MWCNTs), QDs with surface coating, and nanoscale TiO_2 nanocrystals, have been shown to have toxic effects on epidermal keratinocytes and fibroblasts and to alter the gene expression patterns in them.[30,37−39]

The third major route for the NP entry into the body is the gastrointestinal tract (GIT) (Figure 1).[8] Owing to the huge surface area of the GIT, ingestion is probably the most common way of intentional exposure to various NPs. The major process in the penetration of NPs *via* this pathway is transport through the intestinal barrier into the bloodstream. After entering the vascular system, the particles will soon get to the liver, their first-pass organ. Another way of NP distribution from the GIT is the lymphatic system. Lymphatic absorption may give rise to immune response; on the other hand, NPs may affect the mucosal secretory immune function.[8] It has been shown that in the case of oral vaccination, incorporation of antigens into positively charged NPs not only protects the antigen from degradation, but may also increase its uptake by microfold cells (M cells) of the human intestinal epithelium and enhance the association of the antigen with dendritic cells (DCs).[40] Oral administration of albumin−chitosan mixed matrix microsphere-filled coated capsule formulations of Typhoid VI antigen has been demonstrated to induce both antigen-specific systemic and mucosal immune responses.[41] Another example of an enhanced immune response is oral immunization using poly(ethylene glycol)−poly(lactide)−poly(ethylene glycol) (PEG−PLA−PEG) copolymeric NPs as an adjuvant. Oral administration of these NPs encapsulating hepatitis B surface antigen induces strong humoral immunity along with the mucosal (sIgA) and cellular (TH1) immune responses.[42] Since DCs regulate immune homeostasis in the intestinal mucosa and may be the critical targets for ingested particles, the immunogenic potential of TiO_2 and SiO_2NPs has been analyzed. It has been demonstrated that amorphous silica and TiO_2 NPs have strong effects on the activation status of DCs due to activation of the inflammasome with significant IL-1b secretion and upregulation of MHC-II, CD80, and CD86.[43] Thus, after oral intake, NPs may have adverse effects in chronic inflammatory diseases of the intestine. The NP mobility in the gastrointestinal mucus and toxic consequences of the penetration of NPs *via* the GIT depend on their size and surface characteristics. Ingested NPs may interact with ingested food, digestive enzymes, electrolytes, and intestinal microbial flora, *etc*.[8] In addition, pH varies in different segments of the GIT, which may change the reactivity and toxicity of the particles. Moreover, nano- and microsized particles have been found to induce granulomas in different organs and tissues: inorganic particles heterogeneous in nature but homogeneous in size have been identified in liver and kidney biopsies from patients with granulomatosis of unknown origin (10−20 μm in the liver and 6−8 μm in the kidney).[44] Microparticles and NPs of nonbiodegradable inorganic exogenous pollutants have been found in colon tissues affected by cancer and Crohn's disease.[45]

One of the ways for studies of the time course of NP distribution in the body is their direct injection into the bloodstream in animal models.[46] Most NPs are rapidly eliminated from the bloodstream immediately after intravenous injection. They accumulate in tissues of the mononuclear phagocyte system (MPS), mainly Kupffer cells in the liver and macrophages in the spleen.[47,48] For example, starch-coated iron oxide NPs are cleared from the blood within a few minutes after injection.[49] Unless this defense mechanism is evaded, NPs cannot be used in controlled drug targeting and delivery to tissues other than the MPS. Mechanistic studies have demonstrated that NP elimination from the blood is initiated by the adsorption of plasma proteins (opsonins) onto the surface of the NPs followed by phagocytic recognition.[50] This process is strictly dependent on the surface chemistry of nanoparticles. It has been shown that uptake of 100-nm carboxyl-coated polystyrene NPs by different immune cell lines is more rapid than the uptake of amino-coated NPs.[50] The same results have been obtained with carboxyl- and amino-coated quantum dots.[51] At the same time, the mechanism of NP uptake differs for different cell lines. Human macrophages phagocytize polystyrene NPs through interaction of NPs with the CD46 receptor, whereas particle internalization in THP-1 cells is mediated by dynamin II-dependent endocytosis. PMA-differentiated THP-1 cells differ from macrophages and undifferentiated THP-1 cells in that they internalize the particles *via* macropinocytosis.[50] Model studies on rodents have demonstrated that NPs (in the given case, QDs) with a hydrodynamic radius less than 5.5 nm are efficiently removed from the body through renal excretion; this is enabled by zwitterionic or neutral organic coatings precluding the adsorption of serum proteins, which would otherwise increase the hydrodynamic diameter to more than 15 nm and, hence, prevent renal excretion.[52]

Protein adsorption is the initial event that occurs when nanomaterials come into intimate contact with the body. *In vivo* toxic effects of NPs may considerably

vary depending on the multiple parameters of the local environment in the target organs, which determine the changes in the particle size and shape, tissue distribution and concentration, electrical charges, particle-mediated induction of redox activity, characteristics of surface coating, and mechanical stability of the particles.[53] There are numerous data on the interaction of various NPs with blood proteins,[9,11,54−56] formation of complexes between NPs and proteins (the "protein corona"[10,57−59]), and the consequences of this interaction, such as fibrillation of proteins,[56,60,61] as well as specific features of interactions of NP−protein complexes with cells and tissues.[7,62,63]

This review focuses on key aspects of molecular interactions of NPs with living matter, with special emphasis on the molecular mechanisms of interaction of proteins and peptides with NPs (Table 1) in an attempt to predict the possible structural, functional, and nanotoxic consequences of these interactions.

General Principles of Peptide or Protein Behavior on a Planar Solid Interface or on the Surface of a Microparticle. When considering protein−NP interactions, we should discriminate between the interaction of proteins and peptides with nanosized particles proper and their interaction with the surface as a macrostructure. This discrimination is necessary in terms of the differences between the forces that act from an NP surface and an NP as a whole on a protein. The physicochemical properties of planar surfaces, such as the surface energy, polarity, charge, and morphology, play the crucial role in determining their interaction with biomolecules. When considering the characteristics of a surface, one should also bear in mind that common determining factors in various processes on the surface are the properties of its outer atomic layer, which are largely different from those of the bulk material. The outer surface layer is a complex dynamic structure contacting the environment. Here, we focus on those surface characteristics that are known to modulate the interaction with biomolecules, namely the hydrophobic or hydrophilic behavior and the surface charge.

First of all, considering the forces involved in the interaction of proteins with surfaces and NPs, we need to address the question of protein structure, the forces that stabilize it, and their comparison with the forces involved in the protein−surface interactions. If we exclude the phenomenon of intrinsically disordered proteins,[64] which are an exception to the general rules of protein organization, each protein has an intrinsic stability, which is, in thermodynamic terms, the most favorable state of the polypeptide chain, where the entropy of the molecule is minimal. Disturbance of the protein structure should be accompanied by a supply of additional energy to the system to shift it from the "potential hole." These transitions are reversible if the entropy of the "alternative state" is lower than the entropy of the "native state" and irreversible if the entropy reaches the critical maximum value at which the protein is denatured and may form aggregates or fibrils. This phenomenon is the basis for the classification proposed by Norde[65] of all proteins into "soft" and "hard" molecules. From the standpoint of the structure, it is mediated by the interactions underlying the structural stabilization of the protein. The main forces that can stabilize the protein structure are hydrophobic and electrostatic interactions, as well as hydrogen and covalent bonds. Weak van der Waals forces, which play an important role in the stabilization of biopolymers, should also be taken into account. These types of forces and interactions may be involved to different degrees in the stabilization of various levels of protein organization. The secondary structure of proteins represented by α-helices and β-sheets are stabilized by hydrogen bonds alone and hydrogen bonds combined with hydrophobic interaction, respectively. The stabilization of the tertiary structure involves practically all types of forces: hydrophobic interactions form the hydrophobic core of the protein, which becomes more rigid due to covalent disulfide bridges; hydrogen bonds and electrostatic interaction between side-chain residues of adjacent α-helices and β-sheets stabilize the 3D structure. Finally, when the folding of the molecule has been completed, its stabilization is strengthened by van der Waals forces due to various induced polarities (Figure 2A). Although proteins seem to retain intrinsic integrity in the interactions with surfaces at the molecular level, it should be kept in mind that the conformational stability varies in different protein domains.

Let us consider now another aspect of the interaction interface, namely the properties that the surface may impart to the protein. As mentioned above, surfaces as macro- and microstructures have remarkably different features determined by the outer surface layer directly involved in the interaction. One of the most important properties of the surface is its hydrophobicity or hydrophilicity. At the atomic level, the hydrophilicity is determined by the presence of polar functional groups (such as Si−OH and Ti−OH) or under-coordinated metal ions (*e.g.,* Fe^{2+} and Zr^{4+}) in the outer surface layer. The number of such sites determines the degree of hydrophilicity/hydrophobicity, which is one of the determinants of biomolecule interaction. If the surface interacts with a molecule of protein or peptide, hydrogen bonding is important. A globular protein molecule consists of a hydrophobic core surrounded by α-helices and β-sheets stabilized by hydrogen bonds. When the protein molecule approaches an NP, the NP surface replaces water on one side of the molecule. In this case, the nature of the NP surface is important. If the surface is hydrophobic, nonpolar parts that are hidden within the dissolved protein molecule may become exposed to the surface without making contact with water (Figure 2B). In this case, the force of interaction of the protein and the

TABLE 1. Protein–Nanoparticle Interactions

nanoparticle			
type	size (surface properties)	protein/peptide	ref
silica (SiO_2)	6, 9, 15 nm (negatively charged)	human carbonic anhydrase I	96
	10 nm (negatively charged)	human serum and plasma proteins	145
	4, 15, 35 nm (negatively charged)	cytochrome C	97
	20 nm (TEOS-capped)	lysozyme	135
	20 nm (negatively charged)	lysozyme	73
	12 nm (negatively charged)	human transferrin, plasminogen, serum albumin, apolipoprotein A1, immunoglobulins, complement-associated proteins	11
	7.5–82 nm (trimethylsilyl-capped)	bovine serum albumin, bovine fibrinogen	93
	4.2 nm (negatively charged)	β-casein	146
	surface (negatively charged)	lysozyme and succinylated lysozyme	76, 147
		Low-density lipoprotein	148
carbon	SWNT, surface (negatively charged)	soybean peroxidase, subtilisin Carlsberg, trypsin, proteinase K, horseradish peroxidase	101
	carbon black, 14 nm (hydrophobic, negatively charged)	human transferrin, plasminogen, serum albumin, apoliporpotein A1, immunoglobulins	11
TiO_2	21 nm (negatively charged)	apoliporpotein A1, immunoglobulins, complement-associated proteins	11
	20 nm	Aβ(1–42)	134
GSH-CdS	2.6 nm, $GSH_{18}Cd_{60}S_{58}$	GAPDH, ADH, aldolase	100
CdS	9 nm (slightly positive)	hemoglobin	149
CdSe/ZnS	10 nm (capped with various hexapeptides)	Aβ(31–35), Aβ(1–40), Aβ(1–42)	150
	15–20 nm (coated with the polymer with terminal carboxy groups)	human serum albumin	151
	4.4 nm (DHLA-capped)	human serum albumin	127
	2.5 nm (DHLA-capped)	Aβ(1–42)	152
Ag^+(colloid)	31 nm	hemoglobin	56
	15 nm (citrate-coated)	fibrinogen	60
	25–75 nm	bacterial extracellular proteins	153
	16.8 nm (citrate-coated)	lysozyme, human serum albumin, transtyrethrin	154
	19.8 nm (citrate-coated)	bovine serum proteins	57, 155
cationic polysaccharide gel (maltodextrin)	64 nm	bovine calf serum proteins, BSA	156
Au^{2+} (colloid)	12 nm	ubiquitin	95
	5–10 nm (citrate-coated)	insulin, human serum albumin, γ-globulin, histone H3	55
	5, 10, 30, 60 nm (citrate-coated)	BSA	94, 157, 158
	15.8 nm (citrate-coated)	lysozyme, human serum albumin, transtyrethrin	154
	9.4 nm (citrate-coated)	bovine serum proteins	57, 155
	30, 50 nm (citrate-coated)	human blood plasma proteins	9
	5, 10, 20 nm (citrate-coated)	peptide Ac10L	99
	1.5 nm (AET-, BPS- and PEG-capped)	cytochrome C	159
	1.5 nm (mercaptoundecanoic acid-capped)	bovine serum albumin, myoglobin, cytochrome C	160
	10 nm (PEP-capped)	Aβ(1–42)	161
	Surface	fibronectin	71
Fe_3O_4	8 nm (PVA-coated)	transferrin	162
	7.3 nm (TMAOH stabilized)	bovine serum proteins	57, 155
Pd^{2+}, colloid $(NH_4)_2PdCl_6$	3.5 nm	glucose oxidase	163
CoO	7.9 nm (TMAOH-stabilized)	bovine serum proteins	57, 155
CeO	6.7 nm (TMAOH stabilized)	bovine serum proteins	57, 155
polystyrene NP (green carboxylate modified polystyrene)	20–200 nm	bovine serum proteins	164, 165
NIPAM:BAM (85:15, 75:25, 65:35, 50:50)	70, 120, 200 nm (surface charge and hydrophobicity vary with stoichiometry of NIPAM:BAM)	high-density lipoproteins, apolipoprotein A1, human serum albumin, cell cycle regulators, IAAP, Aβ(1–42)	90, 104, 105, 128, 131, 166, 167

Table 1. Continued

nanoparticle			
type	size (surface properties)	protein/peptide	ref
ZrO_2	60 nm (phosphate-grafted)	myoglobin	103
	73 nm (low acidic surface)	bovine serum albumin, lysozyme	77
$CHP/CHPNH_2$ nanogels	30 nm	Aβ(1−42)	130
CdTe	2 nm (TGA-stabilized)	Aβ(1−40)	132
	5 nm (NAC-capped)	Aβ(1−40)	129
Fe_2O_3	15 nm	insulin	61
FePt	15−20 nm	human serum albumin	151
	5 nm (DY-636-labeled and coated with polymer with terminal COO^--groups)	transferrin	58

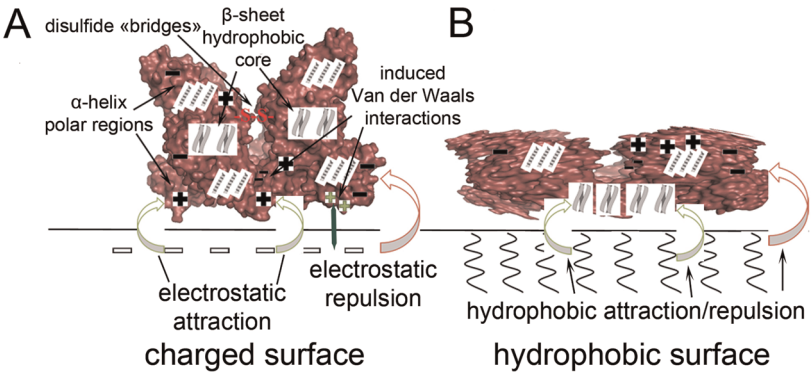

Figure 2. General principles of protein adsorption on surfaces. The forces involved in the interactions of the proteins with the surfaces and their relation to the forces stabilizing the protein structure. The effects of the (A) surface charge and (B) hydrophobicity on the protein adsorption are shown.

surface is only determined by the degree of hydrophobicity of the latter. In the case of hydrophilic (polar) surfaces, the interaction takes place directly through hydrogen bonds with the structures exposed on the surface of the protein. Therefore, the peptide units that may be released when α-helices and β-sheets are disrupted could form hydrogen bonds with the surface. Hydrogen bonds may also form with side-chain amino acid residues, without disruption of the secondary structure. A nonpolar surface is unable to interact through hydrogen bonds. Interaction with it enhances hydrogen bonding of polar parts of the protein molecule in the region of contact. The degree of disruption of the protein structure depends on the balance between the formation and breakage of hydrogen bonds.

Combined simulation and experimental data reported by Anand *et al.*[66] indicate that proteins with a "hard" structure (*e.g.*, lysozyme or RNase A) interact with nonpolar surfaces more effectively than with polar ones. Nonpolar surfaces reduce the energy barrier, simplifying the protein structure rearrangements. Interaction of immunoglobulins with polar surfaces, such as quartz, glass, and cellulose, do not alter its structure,[67−70] but contact of immunoglobulin G (IgG) with the hydrophobic Teflon[70] or hydrophilic stainless steel[68] causes total structure disruption or aggregation, respectively. Simulation of the adsorption of β-sheets on a gold surface indicates that their interaction is expressed in the formation of hydrogen bonds between the protein and the surface and does not corrupt the protein structure.[71] The well-structured molecule of collagen undergoes structural perturbations when interacting with the hydrophilic SiO_2 surface, but it weakly interacts with a hydrophobic derivative of the same surface, which may be explained by its rigid molecular structure.[72] Adsorption of a lysozyme molecule on the polar silica surface is accompanied by structural rearrangements of the protein molecule; the lysozyme conformation is changed due to adsorption, but the native conformation is restored after desorption.[73,74] All these data are in good agreement with the mechanism of the interaction of proteins with polar and nonpolar surfaces described above; however, it is necessary to take into account the characteristics of the protein structure, because the concept of "hard" and "soft" proteins is too qualitative.

Another important property of a surface that determines its interaction with biomolecules is its charge (Figure 2A). If an object with a charged surface is placed into an aqueous solution, an electric double layer consisting of two layers of oppositely charged ions is formed on the surface. The first layer consists of ions adsorbed directly on the surface due to electrostatic forces, hydrogen bonds, coordination bonds, and

van der Waals interactions. The second layer weakly bound to the surface by electrostatic forces is composed of free ions that move in the fluid because of electric attraction and thermal motion but are not rigidly attached to the surface. This second layer is called the diffuse layer. The potential drop across the mobile part of the second layer responsible for electrokinetic phenomena is called the ζ-potential, which is a quantitative characteristic of the charged surface. The ζ-potential is determined by the nature of the surface and, to a considerable extent, the characteristics of the surrounding solution, including its pH and ionic strength. Regarding the interaction with the surface of a protein molecule, the situation is even more complicated. The charge on the surface of a protein molecule is a complicated function of the distribution of amino acid residues in the protein molecule and is unevenly distributed over its surface. In addition, this charge depends on the medium surrounding the protein molecule. For example, it has been found that the modeling of adsorption of lysozyme and α-lactalbumin on a charged surface strictly depends on the pH of the medium.[75,76] Moreover, the interaction of proteins with the charged surface may lead to new "induced" charged regions on the surface of the protein itself. The interaction of the charged surface with the protein changes its ζ-potential. Adsorption of bovine serum albumin (BSA) or lysozyme on aluminum, silica, or titanium microparticles leads to changes in the ζ-potential of the particles, the maximum alteration of the ζ-potential being observed near the isoelectric points of the proteins.[77] In addition, the ζ-potential depends on a number of material-specific features, such as the chemical and crystallographic composition, particle size, crystal lattice, and defects.[78]

Therefore, many factors may regulate the orientation of a protein molecule on the surface, the main ones being charge and polarity determining the attraction or repulsion of the protein molecule. Many protein molecules have an asymmetric spatial shape and may be represented as a prolate ellipsoid.[79] Data on fibrinogen adsorption have shown that the protein may be adsorbed on the surface of mica in two orientations: (i) "side-on," when the fibrinogen molecule is irreversibly adsorbed, with the entire molecule being in contact with the surface, and the adsorption does not depend on the bulk concentration, and (ii) "end-on," when the molecule is reversibly adsorbed, with only the terminal molecule's parts contacting the surface, and the adsorption process depends on the bulk protein concentration.[80] Using the same model for globular lysozyme, Kubiak and Mulheran[76] found that interaction of the protein with a silica surface is determined by electrostatic attraction between parts of the protein and the surface. The model of side-on and end-on oriented particles was applied to lysozyme by Lu *et al.*[81,82] and to BSA by Su *et al.*[83] These authors found that the protein monolayer was thicker and, hence, protein adsorption was stronger, in the side-on orientation than in the end-on one.

Recent studies by Xia *et al.*[84,85] deal with a novel approach to the analysis of nano–bio interactions using the so-called biology surface adsorption index (BSAI). This approach characterizes the adsorption properties of NPs with the allowance for competitive adsorption of small molecules mimicking interaction of individual amino acid residues with NPs. The equation for the BSAI has five terms (biodescriptors) characterizing five molecular forces acting between the NP and the protein: The Coulomb force (electrostatic interaction), London dispersion (hydrophobic interactions), hydrogen-bond acidity and basicity, dipolarity/polarizability, and lone-pair electrons. Application of this mathematical apparatus to experimental data allows us to predict the molecular forces at the nano–bio interface, but we should keep in mind that the approach has been developed for nanosized particles, and extrapolation to a surface should be careful and take into account the differences in its curvature.

Summarizing the general patterns of protein–surface interaction and forces that determine it, we should emphasize that complete understanding of the physicochemical basis of molecular interaction between proteins and the surface is only possible if one combines experimental evidence from both real and model systems with proper mathematical analysis.

When Micro Goes Nano: How Will the Protein–Surface Interaction Change When a Planar Surface Becomes Curved? The size is known to be an important issue in nano–bio interactions.[86,87] When going from the flat surfaces of microparticles to the curved surface of nanoparticles that are comparable in size with protein molecules, nanomorphological factors become the focus of attention in characterizing the interaction. Passing to nanomorphology, we can see that, surprisingly, the surface curvature may affect such an important property of particles as the acidity of the coating molecules. Wang *et al.*[88] reported that the pK_a of the terminal carboxyl groups of the coating polymer on small particles is smaller than that on larger particles. On the other hand, the particle size may affect the surface charge. Simulation studies by Chiu *et al.*[89] have shown that small nanoparticles consisting of hydrophilic material may possess hydrophobic properties but become hydrophilic with increasing size. This behavior arises from the geometrical effect caused by the curvature of the particle–water interface.

The effect of the curvature is at the heart of modulation of the NP interaction with proteins and peptides. Figure 3A shows the comparative sizes of the common blood proteins human serum albumin (HSA) and IgG, QDs, and a hypothetical organic NP. In general terms, the size and, hence, curvature affects the pattern of plasma proteins adsorbed on NPs,[90] but these effects strongly depend on the type of protein under investigation (globular or fibrillar).

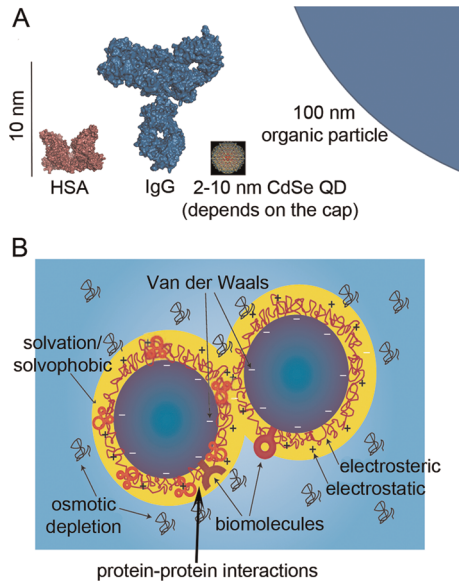

Figure 3. How protein−surface interactions are changed when micro goes nano, and the particle surface becomes curved. (A) Graphical representation of the sizes and structures of some types of nanoparticles (QDs and organic NPs) in comparison with some proteins (HSA and IgG). (B) Protein−nanoparticle interaction forces. The traditional forces for colloidal fabrication (electrostatic, van der Waals, and covalent) and other important interactions (solvation, solvophobic, biomolecular, and depletion) that act when the particles are in a contact with biomolecules. Panel B is adapted with permission from Velegol, D. Assembling Colloidal Devices by Controlling Interparticle Forces. *J. Nanophoton*. 2007, *1*, 012502. Copyright SPIE 2007.

Globular proteins retain their structure as the surface curvature increases, and the functional properties of the proteins are preserved. Indeed, lysozyme,[91,92] BSA,[93,94] ubiquitin,[95] human carbonic anhydrase,[96] and cytochrome C[97] adsorbed on smaller particles retained their native-like structure. In all cases, particles were hydrophilic (10−100 nm silica or 10−20 nm gold NPs). At the same time, adsorption of lysozyme on gold nanorods with the same diameter disturbed its secondary structure and provoked subsequent aggregation of the protein.[91] The mechanism behind the effect of the surface curvature on the globular protein structure can be explained by a simple model. A flat surface provides a larger area for contacts of adsorbed proteins, which results in stronger interactions between proteins and NPs and greater perturbation of the protein structure. However, there are several exceptions, such as RNase A, whose degree of denaturation increases with increasing surface curvature,[98] and chymotrypsinogen, which loses the secondary structure and enzymatic activity on both curved and flat surfaces.[91] Similar results were obtained for a model α-helical decapeptide.[99] When it is adsorbed on a planar surface, it entirely consists of α-helices; gradual changes in the surface curvature (NPs less than 20 nm) results in the appearance of the β-sheet structure. The considerable curvature of 2.4-nm CdSe QDs covered with reduced glutathione strongly affects not only the surface layer of the alcohol dehydrogenase molecule, but also its internal domains, as evidenced by changes in the tryptophan residue fluorescence lifetimes.[100]

Fibrillar proteins, such as fibrinogen, exhibit a different behavior on a curved surface; adsorption of this protein on 15-nm methyl-terminated silica NPs is accompanied by a considerable perturbation of the secondary structure.[93] This can be explained by the side-on orientation of fibrinogen molecules upon adsorption and their subsequent wrapping around the particle. These events lead to the bending of the rodlike fibrinogen molecules and a significant loss of its α-helical structures.

Stabilization of the protein structure and enzymatic activity has also been observed in the case of single-walled carbon nanotubes (SWNTs) and soybean peroxidase. Surfaces with a high curvature may better stabilize proteins in a strongly denaturing environment than surfaces that are flat.[101] The explanation of this phenomenon is that lateral interactions between adjacent adsorbed proteins (the crowding effect) contribute to protein deactivation, these unfavorable interactions being suppressed on highly curved particles, such as SWNTs, compared to flat surfaces. The crowding effect may have other consequences; for example, a high lysozyme concentration and, hence, strong protein−protein lateral interactions on silica NPs result in preservation of the secondary and tertiary structures and enzymatic activity of the protein.[74] Thus, the crowding effect arising from a high protein concentration on a surface with a high curvature may lead to changes in protein adsorption and, consequently, protein structure. As mentioned above, this is accounted for by an increased probability of lateral protein−protein interactions. This phenomenon may affect the structural properties of adsorbed proteins, and it may be accompanied by protein aggregation[91] or formation of fibrillar structures.[102,103]

Another issue that should be considered here is dynamic equilibrium in the interaction of NPs with complex biological fluids, such as serum. In this case, an important factor influencing the protein adsorption on the surface of NPs is its affinity to the surface. Numerous studies on the composition of the protein corona of NPs formed during their interaction with serum[57,59,90,104−106] have demonstrated changes in the composition of the adsorbed protein with time. Initially, NPs mainly interact with proteins whose concentrations in the solution are high. Later, they may adsorb proteins whose concentrations are lower. This is a consequence of different affinities of proteins to the particle surface determined by the chemical properties and size of the particle.

Figure 3B summarizes the data on NP−protein interaction forces. Electrostatic interactions specific for charged surfaces are among the most important forces attracting or repelling charged protein molecules and giving rise to the formation of an electrostatic double layer. The formation of the charged

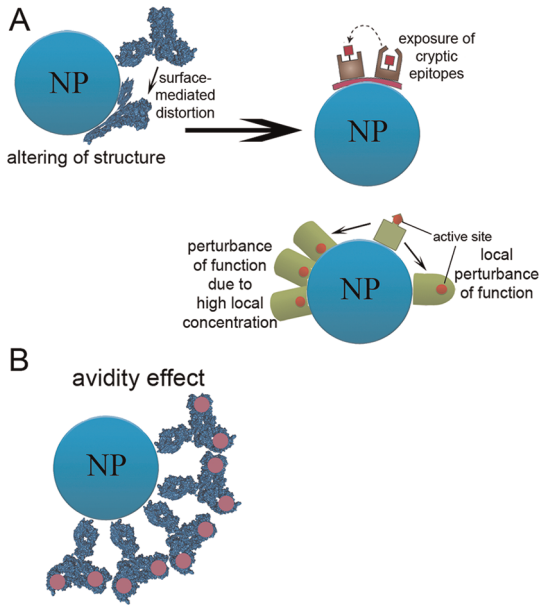

Figure 4. Effects of interaction of a protein with a 15-nm nanoparticle. (A) An altered protein conformation leading to exposure of cryptic peptide epitopes and perturbation of functions caused by the structural effects or a high local concentration. (B) Avidity effects arising from close spatial repetition of the same protein.

double layer on the NP surface, in turn, induces local electrodynamic van der Waals interactions, which may slightly affect the structure and properties of adsorbed protein molecules. Hydrophobic interactions are quite short-range, but they largely contribute to altering the protein structure because, in most cases, interaction with the hydrophobic surface of proteins leads to the strongest structural rearrangements of the protein molecule because of exposure of its inner regions. All these interaction forces may be modulated by the surface curvature. It is well-known that the ζ-potential quantitatively characterizing charged surfaces may vary with changing NP size. An increase in the TiO_2 particle size from 6 to 104 nm leads to a decrease in the isoelectric point of NPs from 6.0 to 3.8.[107] Certainly, this may influence the NP interactions with biomolecules. On the other hand, the curvature affects the formation of a human serum albumin layer completely covering hydrophobic NPs.[108] Finally, a wide variety of protein−protein interactions also affects the structure ofadsorbed proteins.

The interactions on the surface of NPs may lead to different structural rearrangements of the protein molecule. Exposure of the so-called hidden epitopes on the surface of the protein is one of the possible effects (Figure 4A). Changes in the protein structure during adsorption may lead to only partial loss of the native conformation and the formation of a metastable transitional conformation whose free energy is lower than that of the native one. This, in turn, may lead to processes that look like the appearance of new parts of the protein bearing, on their surface, the peptides that are isolated from the environment in the protein's native conformation. These non-native or cryptic peptides may trigger all sorts of biological responses in cells (*e.g.*, cell signaling or uptake of NPs), as well as induce protein aggregation processes, such as fibrillation.[109,110] Moreover, certain proteins after desorption from the surface retain the "new" conformation with an altered stability and altered aggregation patterns, which may lead to potentially dangerous consequences, such as fibrillation. This also applies to proteins involved in molecular recognition or the interaction with receptors. The protein or specific protein domains should maintain their conformation for these types of processes to bring about the expected effect of molecular recognition or specific interaction with their targets.

The issue of the protein structure becomes particularly crucial when considering the functional characteristics of proteins. This is especially important in the case of enzymes, whose catalytic activity is strictly determined by their proper folding and, hence, may be considerably changed or completely lost upon interaction with a surface (Figure 4A). Furthermore, even in the case of preservation of correct folding and integrity of the active site, the correct orientation of the adsorbed enzyme on the surface is an important factor. The interaction of the enzyme with the surface may obstruct the entrance to the substrate channel or interfere with the diffusion of substrates to the active site. In addition, if a protein involved in molecular recognition (, an immunoglobulin or a receptor molecule) exposes the epitope that should be recognized by a receptor or a molecular partner to the solid surface, the related process will be disturbed.[106] The concentration of the adsorbed protein may also play an important role in altering the lateral protein−protein interactions[74] because of the effects of adsorbed proteins on one another's structure. The effects of the adsorption processes on the functional properties of the protein are very diverse. Prediction of the general pattern is difficult because of the huge number of factors that influence these processes. Changes in the structure during the adsorption of lysozyme on negatively charged silica NPs affect its enzymatic activity: a loss of 70% of the initial α-helical structures is accompanied by a loss of 40% of its activity. Further structural changes lead to a complete loss of activity.[92] In this case, the important factor is the orientation of the enzyme on the surface. Lysozyme interacts with model negatively charged surfaces *via* the positively charged amino acid cluster,[76] with the enzyme's active site remaining accessible to the substrate. However, a higher concentration of the protein leads to reaching the saturation state on the flat silica surface, enhancement of lateral protein−protein interactions, reorientation of the protein molecules, and, as a result, loss of enzymatic activity.[111] On the other hand, Wu *et al.*[74] have reported that lysozyme adsorbed on 90-nm

negatively charged silica NPs retains its enzymatic activity only at high protein concentrations. Therefore, the effect of surface curvature is important in the case of lateral protein−protein interactions.

Hulander *et al.*[112] have shown that adsorption of IgG on a curved hydrophilic surface (58-nm negatively charged gold NPs) significantly increases the activation of the C1 complement system, whereas hydrophobicity of the surface blunts this effect. This may result from dense adsorption of IgG on the hydrophilic surface followed by incorrect orientation of the epitope motifs relative to the medium. These data prove the hypothesis on the complex relationship between the surface chemistry/structure and the activity of adsorbed proteins.

Another type of protein behavior is represented by cytochrome C and DNase II, whose adsorption on 4−35 nm negatively charged silica NPs leads to a practically complete loss of activity.[97,113] Similar data were obtained for myoglobin.[103] Adsorption of the protein on phosphate-grafted zirconium oxide NPs leads to losing its enzymatic activity due to significant rearrangement of the myoglobin secondary structure. Interaction of the clotting factor fibrinogen with 15-nm silver NPs retards polymerization of the protein[60] due to significant perturbation of its secondary structure. Measurements of the RNase activity[98] showed that the enzyme retained 90% of its intrinsic activity upon adsorption on 4-nm silica NPs, whereas a 30% net loss was found in experiments with 15-nm particles. This is clearly related to the higher degree of conformational integrity of protein molecules associated with smaller particles.

Nanoparticle curvature and the resultant increased surface area determine an increased number of protein motifs on the "outer" surface of protein exposed to the medium (Figure 4B). This may lead to avidity effects arising from a dense location of actively interacting motifs on the NP surface. Indeed, an increase in the number of folate molecules adsorbed on the surface of NPs leads to a dramatic (up to 170 000 fold) enhancement of the activity of their binding by folate-binding protein (a part of the folate receptor highly expressed on epithelial cancer cells), although the rate of cellular internalization remains unchanged.[114] Functionalization of NPs with the inhibitor of prostate-specific membrane antigen increases the duration of tumor cell exposure.[115] Gubala *et al.*[116] have reported that an increase in the amount of immobilized antibodies leads to an increase in the effective avidity of functionalized silica NPs. There are examples of how multivalency with high avidity can be efficiently used in chemotherapy and immunodetection assays. Because of their unique properties, fluorescent NPs conjugated with antibody are used as a direct label without signal amplification in immunological assays. The use of NPs can only be beneficial if they are efficiently coupled with the detection antibody, have good colloidal stability after conjugation, and the ratio of specific to nonspecific binding (NSB) is sufficiently high. First, after conjugation, the fraction of NP-coupled antibodies should be highly active and available for the reaction with the antigen.[117] Second, minimization of NSB is essential for sensitive detection in NP-based assays.[118] Thus, the methods of NP functionalization and conjugation with antibodies, as well as the choice of the linker moiety, are very important issues. It has been shown that the NP conjugates with IgG[119] or recombinant single-chain (ScFv) antibodies[116] prepared using multivalent poly(amidoamine) dendrimer cross-linkers, exhibit a significantly lower limit of detection, higher sensitivity, and improved stability. The use of dendrimers for conjugation of NPs with antibodies resulted in a significantly higher surface coverage of the active antibody as compared to the use of conjugates with monovalent linkers. As a direct consequence, the increase in the effective avidity significantly outweighs any effect of a decreased diffusion coefficient due to the NPs when compared to that of an antibody labeled with a molecular dye.[116] Moreover, dendrimers exhibit a higher reactivity toward biological material, a feature that could significantly reduce the cost of high-throughput biodiagnostic tests.[119] On the other hand, the increased avidity arising from the high multivalency may lead to nonspecific cell responses, such as nonspecific receptor interaction in nontarget tissues, reducing the efficiency of multivalent targeting. In this connection, it may be important to control the number of the exposed motifs for ensuring the necessary rates of ligand−receptor or antibody−antigen interaction. Indeed, the control of exposed ligand quantity enhances the specific cellular response to elastin-like coated[120] or muscimol-functionalized quantum dots.[121]

To summarize this section, it should be noted that the complexity of the mechanisms of protein and peptide interactions with NPs may give rise to a variety of subsequent effects expressed in rearrangements of biomolecule structure, alteration or even loss of native functions of proteins, and the resulting changes in all homeostatic processes in the cell maintaining its life.

Cooperative Effects in the Interaction of Proteins and Peptides with Nanoparticles. There is much evidence that proteins already absorbed on the surface may enhance or inhibit the adsorption of new protein molecules. Experimentally, this fact is reflected in the sigmoid shape of the adsorption isotherm or an increase in the rate of adsorption. The physical model of the cooperative effects of protein adsorption—positive, negative, and apparently noncooperative—has been described by Chatelier and Minton[122] and further developed by Minton.[122−125] If we consider these phenomena in comparison with Langmuir's classical adsorption theory, the positive cooperative effect is

expressed in an increase in the adsorption isotherm steepness reflecting an increase in the adsorption rate, and the negative cooperative effect is a decrease in the adsorption rate observed as broadening of the adsorption isotherm. Different authors relate the apparent lack of cooperativity to the classical Langmuir adsorption or explain it as a balance between positive and negative cooperativities.

The phenomenon of cooperativity becomes important in the biomedical aspects of interactions of proteins and peptides with NPs, where it may be either beneficial or deleterious to the formation of amyloid structures in neurodegenerative diseases. There are neurodegenerative disorders, such as widespread Alzheimer's, Parkinson's, and Huntington's diseases, whose harmful effect is related to the formation of aggregation-prone proteins and peptides (amyloid β (Aβ) peptides, α-synuclein, and huntingtin with polyQ expansions, respectively) followed by deposition of fibrillar structures.[126] Interaction with NPs may either affect the structure of amyloid-prone peptides, inducing the appearance of β-structures (the initial step of fibril formation) that serve as nucleation centers for fibrillation,[102,127] or bind intermediate units, thereby inhibiting the growth of fibrils or aggregate structures.[128,129] Identification of the possible common patterns of the influence on the formation of amyloid structures and units leading to neurodegenerative diseases is crucial for future applications of nanotechnology to their prevention and treatment.

Nanoparticles as Inhibitors of Peptide and Protein Aggregation. The mechanism of fibril formation by Aβ peptides consists of several stages. First, the native random-coiled Aβ peptide should associate into small (two to six monomers) unstructured soluble oligomers. This step initiates the structural rearrangement in Aβ monomers that lead to the formation of protofibrils, 2- to 5-nm beads with a β-structure, which act as nucleation centers for further elongation of fibrils through the binding of new monomers or other small oligomers.[126]

Ikeda *et al.*[130] reported that small (30-nm) cholesterol-bearing pullulan nanogel particles are strong inhibitors of Aβ peptide aggregation. Moreover, introduction of an additional positive charge (terminal amino groups) into the side chain of the nanogel polymer enhanced the inhibition. The presumable mechanism of the polymer functioning was binding the random-coiled Aβ peptide by nanogel particles, which led to structural rearrangements in the peptide structure and prevention of β-sheet formation. The important role of the surface charge in Aβ peptide binding was observed in the case of *N*-isopropylacrylamide:*N*-*tert*-butylacrylamide (NiPAM:BAM) NPs.[131] Here, the binding of monomers or small prefibrillar oligomers by forming hydrogen bonds changes the equilibrium constants of fibril formation and hinders the formation of critical nuclei and elongation of the

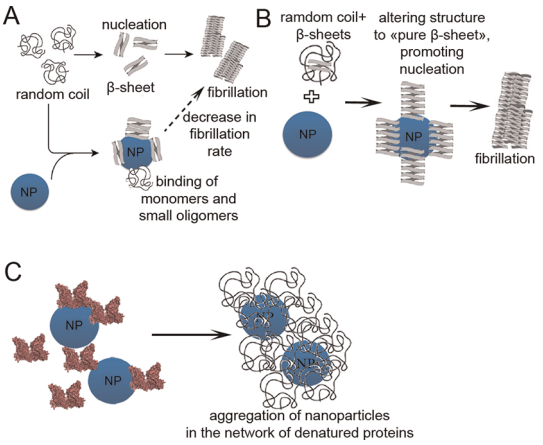

Figure 5. Cooperative effects involved in the interaction of proteins and peptides with nanoparticles. (A) Nanoparticles as the centers of self-association or fibrillation of proteins and peptides. (B) Nanoparticles as the inhibitors of self-association of proteins. (C) Self-assembly of nanoparticles on biopolymers serving as templates.

fibrils (Figure 5A). This mechanism was confirmed by a later study with the same NPs and amyloid protein related to type II diabetes mellitus.[128] In this case, an increase in the hydrophilicity of NPs is accompanied by enhanced inhibition of the nucleation stage of fibril formation. In all cases studied, the mechanism of the NP inhibitory effect is strongly dependent on the extent of their hydrophilicity, which is not surprising in view of the importance of hydrogen bonds in the formation of β-structures. Competition for hydrogen bonding during the interaction of hydrophilic NPs and monomers or small oligomers leads to the formation of complexes between them and inhibition of the nucleation of fibrils.

Similar data have been obtained in experiments with CdTe/CdSe quantum dots capped with various chemicals. *N*-Acetyl-cysteine CdTe QDs of 3−5 nm inhibit the formation of fibrils by Aβ peptide by binding with active oligomerization centers at the C-terminus of the growing fibril and retarding fibrillogenesis at all stages from nucleation to fibril elongation through hydrogen bonding.[129] Dihydrolipoic acid (DHLA)-capped CdSe quantum dots act in the same way except that they bind only protofibrils and fibrils and inhibit the elongation stage. Another effect of CdTe quantum dots capped with thioglycolic acid (TGA) was observed recently.[132] Although NPs prevent the formation of Aβ peptide fibrils at all stages by preferentially binding oligomers or protofibrils, the authors assumed a different mechanism of interaction. Formation of protofibrils is accompanied by formation of β-structures strongly stabilized by hydrogen bonds. Considering that there was already strong H-bonding in the oligomers, the authors assumed that the TGA molecules could hardly compete with these H-bonds, which were strengthened by a specific conformation of the peptide. They hypothesized that, in this case, van der Waals forces,

short-range but powerful interactions related to a high electron density of atoms in semiconductor nanocrystalls, play an important role in binding oligomers. This behavior is very similar to that of proteins, which can also bind amyloidogenic oligomers.[132] The authors confirmed this hypothesis by FTIR data, which did not show extensive formation of H-bonds between the NPs and oligomers.

Nanoparticles as Nucleation Centers in Amyloidogenesis: Possible Implications in Neurodegenerative Diseases. One more effect of NP interaction with amyloid-prone proteins and peptides is their ability to promote aggregation of biomolecules or increase the fibrillation rate. In some cases, interaction between NPs and common blood proteins may lead to protein fibrillation. The protein molecules that have already been adsorbed undergo structural perturbations, which initiate further interaction of proteins from the bulk solution with NPs and promote their structural rearrangements. This may result in accumulation of denatured or amyloid-prone molecules on the surface of NPs (Figure 5B).

Adsorption of lysozyme on gold NPs significantly alters the protein secondary structure. This, in turn, leads to the formation of protein aggregates on the surface of NPs, which induces the interaction with other protein molecules from the solution and their subsequent unfolding and denaturation leading to the formation of large protein aggregates surrounding the NPs.[133] Similarly, the interaction of phosphate-grafted zirconia NPs with myoglobin results in drastic rearrangement of the protein structure (both secondary and tertiary) and formation of prefibrillar protein aggregates. The morphology and sizes of prefibril-like aggregates remain stable, whereas the formation of long, straight fibrillar aggregates has never been observed.[103] On the other hand, interaction with nonfunctionalized zirconia NPs, as well as with NPs of silica and cerium oxide, does not affect the nucleation stage of amyloid formation by Aβ (1−42) peptide. However, titanium oxide efficiently promotes this process.[134] This suggests that the particle size is less important for the ability to promote Aβ fibrillation than are the composition and surface characteristics. Another behavior of cerium oxide NPs was observed in their interaction with β_2-microglobulin,[102] whose fibrillation is the main cause of hemodialysis-related amyloidosis.[126] These NPs, as well as CdSe QDs and multiwalled carbon nanotubes (MWCNTs) and NiPAM:BAM NPs, increase the nucleation rate during fibrillation. There is an exchange of protein between the solution and the NP surface, with β_2-microglobulin forming multiple layers on the particles surface, thereby increasing the local protein concentration and promoting oligomer formation. So, the size and surface chemistry determine the strength of the effect. In the case of the NiPAM:BAM copolymer, the effects obtained with 70-nm and 200-nm NPs were compared. The results indicated that smaller and more hydrophilic particles more efficiently promoted nucleation,[102] and 200-nm and hydrophobic particles were less efficient. This effect of size on nucleation may be explained by differences in the NP surface curvature. Apparently, small NPs with a high surface curvature cause greater structural rearrangements of the β_2-microglobulin molecules. In addition, the high local protein concentration and correspondingly high degree of protein−protein interactions may explain how NPs facilitate fibril formation. Their modulating effect promotes the formation of dimers and small oligomers of this protein, which serve as nucleation centers for subsequent fibrillation. This suggestion is supported by an increased fibrillation rate of human serum albumin in the presence of DHLA-capped CdSe quantum dots.[127]

Thus, no general pattern of the effects of NPs on fibrillogenesis has been found. The current methods for evaluating the effect of NPs still rely on the case-by-case approach, which is laborious and time-consuming due to the diversity of NPs. However, the high toxicity of NPs should be borne in mind. Their use is limited because of their ability to easily penetrate the blood−brain barrier and induce the formation of amyloid-like structures typical of neurodegenerative diseases.

Self-Assembly of Nanoparticles on Biopolymers Serving As Templates. As we saw above, there are numerous data on the effects of NPs on protein and peptide structure leading to various consequences, such as inhibition or promotion of aggregation/fibrillation. However, biomolecules may also promote NP aggregation. This phenomenon is mediated by protein−protein interaction, which may occur at a high protein concentration on the NP surface. In some cases, protein molecules form bridges between NPs (Figure 5C).

There are two possible mechanisms of bridging. The first one is based on structural perturbations in protein molecules resulting from interaction with NPs. In this case, an unfolded or partly folded protein molecule already adsorbed on the surface interacts with another molecule from the solution and provokes its unfolding. Subsequent stages lead to accumulation of unfolded protein molecules on the surface of the given NP and adjacent particles. This has been observed during the interaction of gold NPs with lysozyme.[133] On the other hand, protein−protein interaction may occur without structural perturbation and may be entirely determined by electrostatic interaction between protein molecules. Interaction of silica NPs with lysozyme leads to pH-dependent protein-mediated NP aggregation.[135] This phenomenon may be explained by different charges on the protein and NP surfaces. In the pH range between the isoelectric points of the silica surface and lysozyme, the adsorption of protein is causing NP aggregation. This is explained by the excess surface charge on the lysozyme

molecule combined with its low dipole moment, which leads to redistribution of the positive charge over the surface of the protein molecule and interaction with the negatively charged surface of silica. There is evidence of fibrinogen-mediated aggregation of silica NPs; in this case, however, the particle size plays an important role.[136] Lower fibrillation rates were observed for particles with larger surface areas. This may be explained by the rodlike architecture of the fibrinogen molecule, which may wrap around a large particle without interacting with other fibrinogen molecules.

Interaction of common blood proteins with citrate-coated gold NPs was observed by Lacerda *et al.*[55] One of the main findings was a very slight effect of adsorption on the protein structure; however, the NPs were coated with the protein, which dramatically increased their size and caused their aggregation. This should be taken into consideration when NPs are used as therapeutic agents.

CONCLUSIONS

Implications of Nanoparticles in the Protein Interactome. Better understanding the processes underlying the interaction of NPs with biomolecules is the basis for biological and medical applications of NPs. The physicochemical properties of NPs, their size, and the chemistry and topography of their surface determine a wide range of their effects on biological systems. The specific properties of biomolecules further complicate the picture.

The main potential consequence of the interaction of proteins and peptides with NPs is the alteration of the biomolecule structure. This effect strongly depends on the surface characteristics and intrinsic protein stability. Structural alterations may affect the protein functions, which are closely related to the structure. For example, the enzymatic activity may be affected, which is crucial for biological systems. Structural perturbation is also undesirable in the case of biological recognition. An altered receptor or antibody structure may lead to the loss of cell signal transduction or immune response, respectively. On the other hand, interaction of a protein or peptide with an NP and the resultant structural perturbation may lead to the loss of the ability to interact with other proteins and, hence, form biologically active complexes. Thus, NPs may change the normal interaction patterns, thereby disturbing cell homeostasis. However, it is as well to remember that NP-induced changes in the structure of the adsorbed protein may have a positive effect. This is, for example, the inhibition of fibrillation of peptides and proteins involved in the pathogenesis of neurodegenerative diseases. On the other hand, NPs may, conversely, promote this pathological process.

Oxidative stress, which has not been dealt with in this review, is another important effect of protein–NP interaction. It may be provoked by various factors, such as oxidative damage of proteins by a reactive surface, generation of particle-derived reactive oxygen species, and depletion of the antioxidant pool.[137–141] This diversity of effects may lead to various cell responses, including cell death. It is worth remembering that the response of the immune system to NPs is assumed to be related to the generation of bioactive protein–NP adducts. Protein adsorption may in fact lead to the exposure of receptors involved in various pathways of inflammation.[137] At the same time, NPs may enhance the antigenicity of conjugated weak antigens, thus serving as an adjuvant.[142]

In the light of these considerations, understanding the mechanisms and factors of NP–protein interactions is necessary for prospective diagnostic and therapeutic applications of NPs. Various techniques should be used to accurately and carefully modify NP properties, which is crucial in terms of the structures and functions of the adsorbed proteins[143] and, hence, the NP toxicity mediated by the alterations of these proteins. Although great progress has been made in the synthesis, characterization, and applications of NPs, a number of key challenges remain unresolved and need to be addressed in order to promote new medical applications of NPs as delivery systems and imaging tools.[144]

It is worth mentioning that the risk assessment procedure should include numerous types of molecular, cell culture, and animal model tests to identify the potential hazards of nanoparticles before they could be used in biomedical applications. These tests should begin with physicochemical characterization of functionalized nanoparticles, providing information about their structural properties, stability, and susceptibility to biodegradation; the main parameters to be estimated are the particles size, hydrodynamic diameter, zeta potential, and electron microscopy structural parameters. The patterns of variation of nanoparticle properties in biological fluids and tissues should also be studied.

Further tests should provide information about the molecular and cell toxicity of the nanoparticles upon their interaction with biomolecules, human cells, and tissues. These tests should include the spectroscopic analysis of the structures of proteins adsorbed on the particle surface, specific fluorescent assays of cells viability, immunochemistry, and enzymatic activity tests, as well as genomic, proteomic, transcriptomic, cellular metabolism, and general toxicity analyses in cells and in animal models.

Although the final conclusion about the potential hazards of the use of nanomaterials in life sciences will be provided by the last series of specific nanotoxicity tests, the current knowledge on molecular interactions between proteins and NPs provides a fundamental basis for understanding the molecular mechanisms

of nanotoxicity which should help in developing approaches to its reduction, thus extending the possibilities of therapeutic applications of NPs.

Hence, a detailed understanding of the physicochemical aspects of interaction between proteins and NPs is the basis for reduction of nanotoxicity and further therapeutic applications of nanomaterials.

Conflict of Interest: The authors declare no competing financial interest.

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