



Molecular modeling in structural nano-toxicology: Interactions of nano-particles with nano-machinery of cells[☆]

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ABSTRACT

Over the past two decades, nanotechnology has emerged as a key player in various disciplines of science and technology. Some of the most exciting applications are in the field of biomedicine – for theranostics (for combined diagnostic and therapeutic purposes) as well as for exploration of biological systems. A detailed understanding of the molecular interactions between nanoparticles and biological nano-machinery – macromolecules, membranes, and intracellular organelles – is crucial for obtaining adequate information on mechanisms of action of nanomaterials as well as a perspective on the long term effects of these materials and their possible toxicological outcomes. This review focuses on the use of structure-based computational molecular modeling as a tool to understand and to predict the interactions between nanomaterials and nano-biosystems. We review major approaches and provide examples of computational analysis of the structural principles behind such interactions. A rationale on how nanoparticles of different sizes, shape, structure and chemical properties can affect the organization and functions of nano-machinery of cells is also presented.

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“As crude a weapon as the cave man’s club, the chemical barrage has been hurled against the fabric of life—a fabric on the one hand delicate and destructible, on the other miraculously tough and resilient, and capable of striking back in unexpected ways.” — Rachel Carson, In Silent Spring, (1962), 297.

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1. Introduction

In his epochal speech of 1959 [1] Richard Feynman, not only formulated new concepts in nanotechnology and, highlighted its significance for science and research, but also forecasted several new directions in nanobiology, nanomedicine and nanotoxicology. He introduced the idea of nanomaterials and alerted us: “At the atomic level, we have new kinds of forces and new kinds of possibilities, new kinds of effects. The problems of manufacture and reproduction of materials will be quite different. I am inspired by the biological

phenomena in which chemical forces are used in a repetitious fashion to produce all kinds of weird effects.” Explosively fast development of a huge diversity of nanomaterials and nanodevices has led to their applications in different frontier technologies, such as filtration, electronics, cosmetics, energy, medicine, chemicals, coatings and catalysts. As a result, the market for a number of nanomaterials – titanium dioxide, zinc oxide, silicon oxide nanopowders, carbon-based nanoparticles (Fig. 1), nanofibers, nanosilver, nanoclays, quantum dots and nanoporous materials – has already been established. There are emerging market opportunities for graphene and nanocellulose. According to a recent detailed report from Future Market, Inc., [2], the 2011 worldwide production of nanomaterials of more than 270,000 tons (a tenfold increase from 2002) is conservatively estimated to reach the production volume of more than 350,000 tons in 2016. Now, when the era of nanotechnologies and nanomaterials has already commenced – what did we learn about interactions of man-made nanomaterials with nano-structures of cells and biofluids in our body?

Almost a century and a half ago, Charles Darwin, in his unifying and visionary book “On the Origin of Species,” concluded that all traits of organisms have been optimized and improved to near perfection by natural selection. Among those traits are evidently the sizes and 3D-organization of cells, intracellular organelles, other bio-molecular machines and macromolecules. It is currently believed that since the origin of the first metazoans over 600 million years ago, cell type diversification has been driven by micro-evolutionary processes at the

population level, leading to macro-evolution changes above the species level [3]. According to principles of “noise biology,” the behavior of intracellular assemblies is dependent on the inherent stochastic fluctuations in molecular transitions or interactions [4]. These fluctuations are especially significant in small systems where the magnitudes of the fluctuations may “approach or exceed the mean value of the molecular population.” This is particularly important for nanomedicine and nanotoxicology where “small synthetic and biological systems are bound by their size to reside in environments of large fluctuations.” Assuming that biological endogenous nano-structures and artificial nano-materials (Fig. 1) are molecular assemblies of similar size, what kind of effects – stimulation, interference, disorganization, chaos – can we expect from their interactions in the body?

Recent focus in the field of nanotoxicology has shifted towards understanding the details of unintended or undesired interactions of nanomaterials with biological molecules. There has been a surge in the application of *in silico* based methods and approaches in investigating the structural aspects of toxicity as they relate to the structure and dynamics of nanoparticles [5], and molecular details of their interactions with biomolecules, including lipids and membranes [6–9], peptides and proteins [10–13], and nucleic acids [14–16]. Several excellent recent reviews summarized a diversity of experimental and theoretical aspects of computational modeling of nanoparticles and nano-bio interfaces [17–19]. In the current review, the emphasis is on a rationale for the use of computational approaches to understand

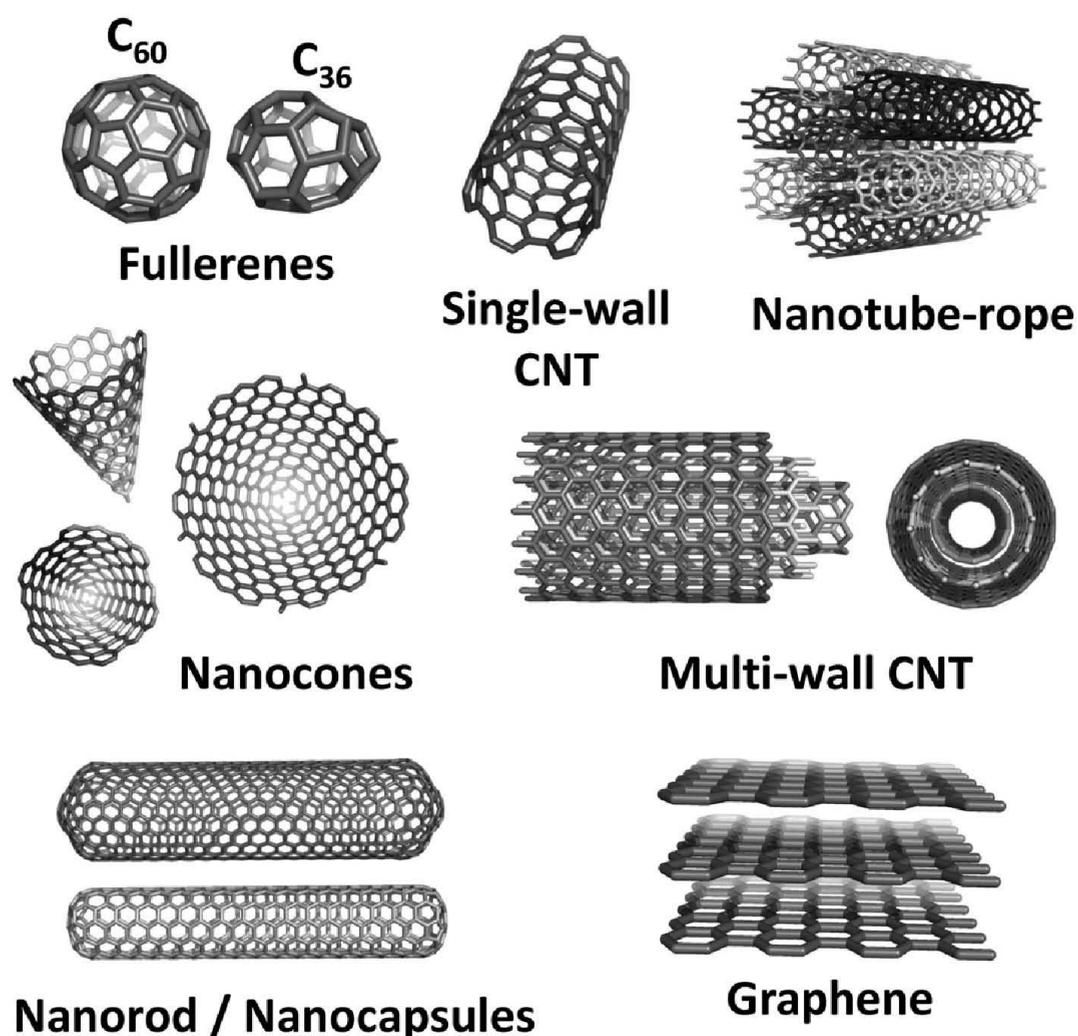


Fig. 1. Carbon based nanoparticles. Representative 3D structures of different types of carbon based nanoparticles. The structures of nanoparticles are rendered as sticks.

how nanoparticles of different size, shape, structure and chemical properties can affect the organization and functions of biomolecules and nano-machinery in cells. A brief summary highlighting the possible scenarios of nanoparticles interactions and their interference with biomolecules in cellular environment with relevant examples is also discussed.

2. Physical interactions of nanoparticles with cellular components

The sub-cellular organelles, macromolecules, and other biological structures (mitochondria, complexes like ribosomes, nuclear pore) have evolved into well-organized sizes and shapes to carry out their intended roles in cells. The geometric, structural and chemical properties attained by these molecular arrangements define their precise function and interactions inside cells. Size-wise most of these biological assemblies of molecules and their complexes are within the nanometer (nm) range, similar to most common types of nanoparticles. Because of this co-incidence in size, the engineered nanoparticles, that are purposely designed and manufactured for therapeutic and diagnostic use or inadvertently get into the body, and navigate through biofluids, tissues or targeted cells, should inevitably interact and likely interfere with a wide variety of biological nano-objects of different complexity from endosomes, proteasomes, nuclear pores, ribosomes, and mitochondria to solubilized or membrane bound enzymes, receptors, channels, structural proteins. While the initial descriptive toxicological studies of nanoparticles largely neglected these interactions, the focus of recent mechanistic work has shifted to understanding the structural and molecular details of bio-nano-interfaces using different methodologies, including computational approaches [20]. The current literature on computational approaches to molecular description of interactions of nanoparticles with proteins and other biomolecules has been discussed in several recent reviews [17–19]. The goals of this section are: i) to establish the link between the sizes of nanoparticles and the important structural properties of the types of intracellular biomachinery and macromolecules, and ii) develop more profound quantitative understanding of the essential details relevant to interactions of nanoparticles with these structural/functional intracellular units.

2.1. Interaction of nanoparticles with proteins

Proteins are crucial for cell's existence and functions, as they virtually participate in every process performed within the cells by interacting with small molecules – ligands, substrates – and other proteins as well as nano-structured biomembranes. As linear polymers built from sequences composed of a relatively small number of different amino acids (~20), proteins form highly diversified 3D-nano-assemblies. Thus, nanoparticles with their different architectures and surfaces have the potential to interfere and/or alter the native functions of proteins.

Notably, the 3D and 2D structures of the ligand binding pockets and catalytic sites in different proteins, despite their diversified functions, size-wise all have dimensionality ranges similar to those of nanoparticles. Indeed, several types of NPs, such as fullerene (C₆₀) and SWCNTs, can bind and suppress the activity of enzymes as exemplified by HIV-1 protease (HIV-1P) [21–23] and glutathione-s-transferase (GST) [24,25]. Based on a docking simulation study, Wudl and Wilkins et al. [26–28] speculated that the core of C₆₀ is enclosed in the HIV-1P substrate binding site, composed primarily of hydrophobic amino acid residues (Gly, Phe, Ile) and a highly conserved catalytic active site composed of Asp-Thr-Gly residues. Subsequent molecular and structural details of interactions of HIV-1P with fullerene and SWCNT, using computational approaches [21,29], revealed that fullerenes as well as the SWCNTs with a diameter of ~0.7 – 1 nm effectively interact with the hydrophobic ligand binding site of the enzyme (Fig. 2A(i)). Further, based on molecular dynamics simulation studies, it was proposed that

the binding of SWCNT, prevents the enzyme flaps at the active site from opening up to bind polypeptides for cleavage, thus blocking its activity [30,31]. Considering, the combination of hydrophobic and electronegative interactions at the site, NPs, that are either neutral or positively charged, have the potential to interact with it. In fact, this is being exploited by several investigators to develop fullerene-based inhibitors of HIV-1P for AIDS therapy [22,26–28,32,33]. In addition to HIV-1P, the aqueous suspensions of fullerenes were also found to inhibit the activity of GST [24,25]. Docking simulations predicted that C₆₀ binds to a cleft at the GST dimer interface (Fig. 2A (ii)), which is normally occupied either by glutathione or other substrates. Blocking this binding site in GST and suppression of GST function could lead to cytotoxicity and increased oxidative stress in cells. In a recent review, Zuo et al. [34] summarized interesting studies on the interactions of proteins with carbon nanotubes (CNTs) and fullerene derivatives (fullerenols, metallofullerenols) that may be essential for understanding of the molecular mechanisms of nanotoxicity. In particular, specific hydrogen bonding as well as non-specific electrostatic and hydrophobic interactions of a metallofullerenol-based inhibitor, (Gd@C82(OH)22), have been shown to be responsible for the suppression of matrix metalloproteinases (MMPs) thus suggesting a therapeutic potential in cancer therapy [35].

The NPs also exhibit similarity in dimensions to channels or pores formed by membrane proteins involved in transporting molecules and ions. For example, a typical pore of ion channels, should have a diameter between 0.3 – 1.2 nm (Fig. 2B). The closed versus opened state of a pore and its specificity for the conductance of different ions are strictly regulated by nano-sized arrangements within the protein assembly [36,37]. In addition to regulating the diameter of the pore to discriminate ions based on their size, each ion channel also contains a selectivity filter with several negatively-charged oxygen atoms from backbone carbonyl groups, which mimics spatial arrangement of water molecules around each ion [36,37]. The interplay between the diameter of the pore as well as the precise number and arrangement of negative charges around the filter allows a channel to render high selectivity for different ions with similar charge. Interestingly, NPs – like C₆₀ and SWCNT – are capable of blocking ion channels [38]. Molecular docking studies by Park et al. [38] showed that fullerenes as well as SWCNT with an average diameter between 0.7 and 0.9 nm can “sit” on the top of a potassium channel (KcsA) and block the entrance for K⁺ ions. The binding to the channel is not only affected by the size but also by the shape of the nanoparticle. An open ended SWCNT as compared to a capped SWCNT was found to be more efficient in blocking the K⁺ channel, probably due to its extended interaction with the selectivity filter. Atomistic molecular dynamics (MD) simulation studies by Kraszewski et al. [39] suggested that the binding of C₆₀ fullerenes to different K⁺ channels (KcsA, Kv1.2 and MthK) mainly depends on their size and hydrophobicity. Therefore, the binding of nanoparticles with a diameter of 0.9 – 1.3 nm – typical of a number of carbon-based NPs (Fig. 1) – to these pores can effectively block ion transport across the membrane and lead to toxicity [38,39].

Not only the similarity in shape and size, but sometimes the physical properties of NPs can parallel and mimic features of structural proteins, which play a critical role in maintaining cell shape, size, morphology and motility. For example, many studies have shown that actin filaments (one of the three components of cytoskeleton) and focal adhesion structures are altered in cells treated with CNTs [40–43]. In particular, actin filaments were found to be altered and redistributed in cells treated with SWCNTs [44]. Molecular dynamics simulations demonstrated that SWCNTs preferentially bind actin, whereby the essential molecular details mainly include stabilized hydrophobic and π - π interactions [44,45]. The similarity between actin filaments and CNTs in their length (~3 – 18 μ m vs 1 – 15 μ m) and diameter (~8 nm for actin vs MWCNTs and CNT nanoropes with diameters \leq 10 nm) supports the hypothesis that non-specific

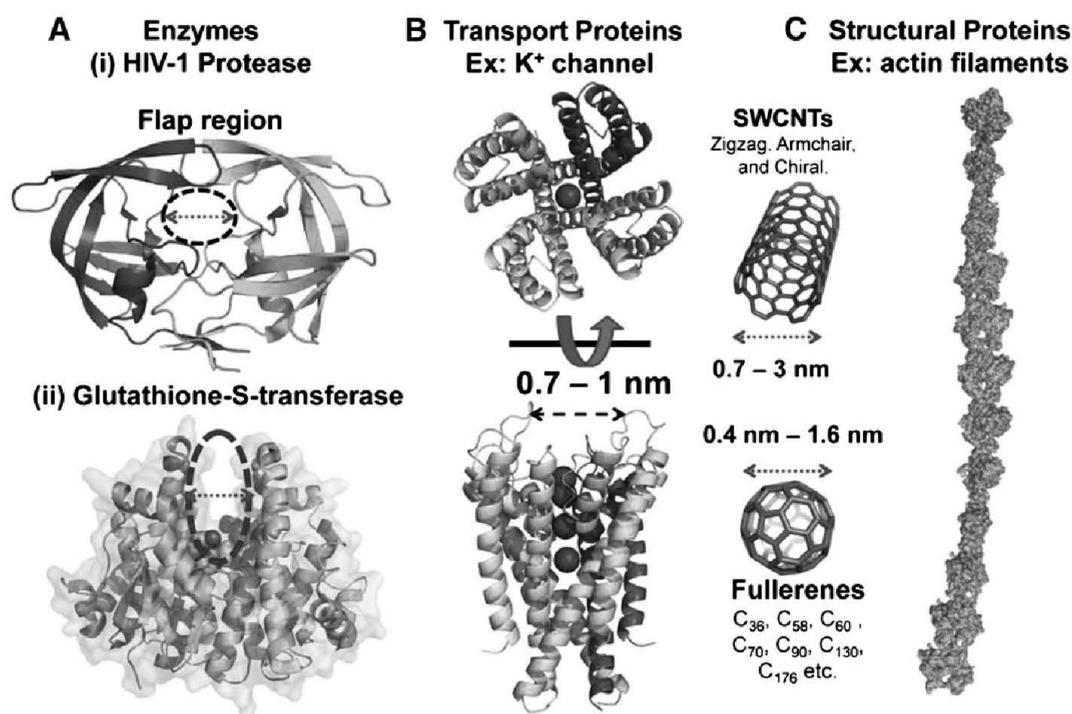


Fig. 2. Examples of different types of proteins in cells. Three dimensional structure(s) of representative members of (A) Enzymes – HIV-1P and GST illustrating their active ligand binding sites, (B) Transport proteins – top and side views of potassium channel, showing the pore for K⁺ ions, and (C) Structural proteins – the stiff and resilient structure of actin filament. The structures of proteins in (A) and (B) were colored by chains and rendered as a cartoon. Dotted arrows and circles highlight the similarities in structural properties between proteins and nanoparticles.

(hydrophobically driven) interactions of SWCNT, with the surface of actin monomers are sufficient for their stable associations in cells. Although MWCNTs are rigid in comparison to SWCNTs, a bundle of ~13 – 20 SWCNTs with a diameter of 2nm arranged in 2 layers to form a 'nanorope' (Fig. 1) of outer diameter ~8 – 10 nm, could still exhibit similar mechanical properties (flexibility and fluctuations) as actin filaments (Fig. 2C). Similarly, the shape, size and physical property of CNTs also parallel the structural and physical properties of intracellular microtubules, despite remarkable differences in their chemical compositions [46–48]. Indeed, both microtubules (MTs) and CNTs are hollow cylindrical structures. The hollow cylinder in MTs is formed by packing protein (ex: tubulin) into a helical lattice, while a hexagonal lattice of carbon atoms defines the cylindrical structure in CNTs. Similar to MTs, arranged as bundles in cells, CNTs can also form bundles, referred to as 'nanoropes', by packing SWCNTs tightly in a hexagonal order through van der Waals interactions (Fig. 1). This not only allows CNTs to be stiff, but also to be highly resilient similar to MTs. These features suggest that CNT, can interfere with microtubule functions in cells, as indeed has been reported by several groups [47,49]. NPs were found to be associated with microtubules and centrosomes [49]. Further, such interactions were also shown to induce mitotic spindle disruption, chromosome breakage/fragmentation, no/multinucleate cells and mutagenicity [49–53]. The interference of NPs with the components of cytoskeleton can also indirectly affect the functioning of organelles, as their proper organization, coupling with other organelles, distribution and localization in cytosol is influenced by cytoskeleton and is central to multiple cell survival and cell division mechanisms.

2.2. Interaction of nanoparticles with biological nano-machinery

Cells contain numerous specialized nano-sized structures – known as 'biological nano-machinery' – that function in harmony to carry out specific functions. Interfering with the natural rhythm of

the nano-machinery either entirely, or with one or more of its components leads to a faulty system.

Some of these assemblies are complex and are made up of several proteins and/or nucleic acids assembled together in an organized fashion [54,55]. For example, the ribosome is a molecular complex composed of proteins and RNA with specific and precise locations for accommodating tRNA, mRNA, and newly synthesized polypeptide chains within the cavities that have a diameter of ~2nm. Due to their comparable shape, size and chemical properties, NPs, like fullerenes, CNTs and others, can bind at these sites, inhibiting or arresting protein synthesis.

Other multi-protein intracellular assemblies – like exosomes and proteasomes – are involved in degrading obsolete RNA and proteins, respectively. They contain an interior hydrophobic hollow cavity surrounded by catalytic site residues that are capable of cleaving and degrading the respective biomolecules (Fig. 3A). The specificity for cleaving different molecules originates from the type of catalytic site residues in the interior of the channel (Fig. 3A). The diameter of hollow core in these assemblies however ranges between ~1.2 to 5 nm. Thus hydrophobic carbon based NPs (Fig. 1), like fullerenes, nanorods and CNTs having comparable diameters, size and shape, may effectively bind and occupy these sites and potentially interfere with the functioning of the nano-machinery.

Intracellular organelles – mitochondria, nucleus, endoplasmic reticulum – are specialized structures whereby their components are separated from cytoplasm by a membrane. If NPs get in close proximity to these organelles, their physical interactions may cause disruption of their normal functions in cells [56–60]. For instance, mitochondrial damage is believed to be one of the likely mechanisms by which NPs cause toxicity to cells by inducing oxidative stress via disruption and discoordination of normal electron transport by respiratory complexes. Further, the voltage-dependent anion channel (VDAC), located in the outer mitochondrial membrane, is the major component of its membrane and acts as a gatekeeper for the entry

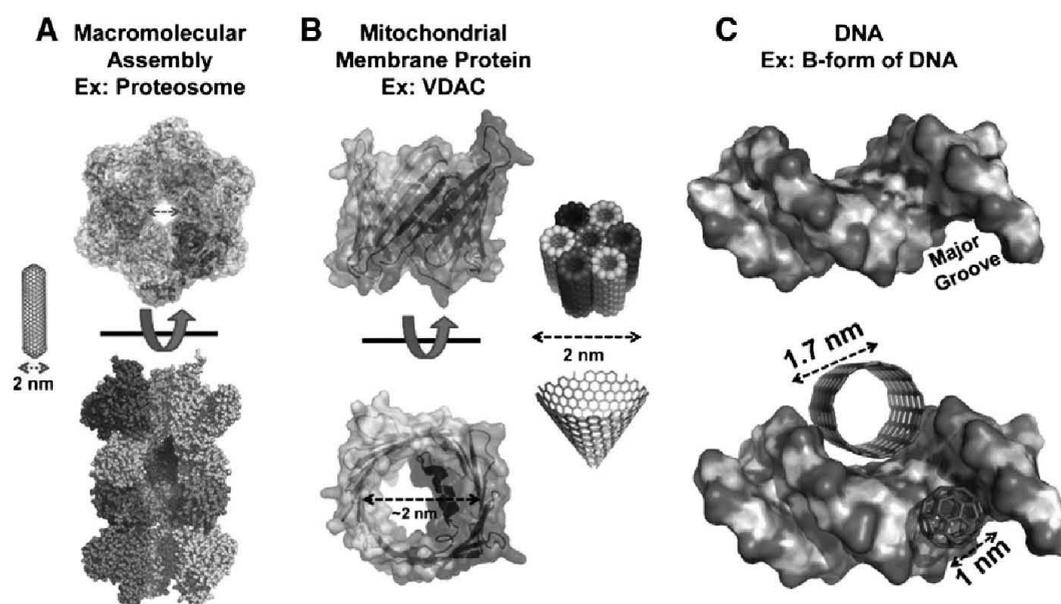


Fig. 3. Comparison between nanoparticles and biological components. Similarity in sizes between the carbon based nanoparticles and 3D/2D structural features of different biological components in cells including (A) macromolecular assemblies of proteasomes, (B) mitochondrial outer membrane voltage-dependent anionic channel (VDAC), and (C) DNA. Cartoon representation of the top view and side sectional views of (A) proteasomal complex with a central channel, (B) mitochondrial membrane protein, VDAC, with a pore that transports ions and molecules in and out of mitochondria, and (C) DNA depicting the major and minor grooves. The predicted binding poses of SWCNT and fullerene, C_{60} , on DNA are also shown in (C). The similarities in the diameter between different carbon based nanoparticles and specific structural features in biological components are indicated by dotted arrows and colored differently in each case.

and exit of mitochondrial metabolites. VDAC, spanning the membrane, contains a central channel with a diameter of ~ 2.6 nm. Compared to its entrance, the size of this channel is constricted in the middle (~ 1.8 nm vs 2.6 nm) to render specificity for transporting different anions and contains a set of positively charged, lysine residues, to preferentially interact with negatively charged anions (Fig. 3B). As mitochondria play a major role in maintaining the ATP/ADP balance in cells, binding to and blocking of this channel by NPs having similar dimensions and/or a negative charge, can cause ATP-ADP imbalance in cells.

2.3. Interaction of nanoparticles with DNA

DNA coated NPs are often used for theranostics purposes in medicine including high-throughput and automated SNPs genotyping [53], detection of ions in body fluids, improved methods for decoding DNA, and gene therapy. Despite the overall negative charge of DNA, a strong binding to carbon based uncharged NPs, like fullerenes, SWCNTs and MWCNTs (Fig. 1), has been reported [16,61–65]. To design new nanomaterials with reduced geno-toxicity, it is critical to understand the mechanisms of interactions between DNA and NPs. Several *in vitro* and *in vivo* studies demonstrated the potential of NPs to interfere with processes involving DNA [48,50–53]. The physical interaction of NPs with DNA may lead to potentially negative impacts on its structure, stability, and biological functions. Because possible clinical outcomes associated with DNA damage, particularly cancer, may require long times to become detectable *in vivo*, computational approaches to studies of interactions between DNA and NPs may be very useful.

Considering that the diameter of fullerenes and CNTs is close to the sizes of the major and minor grooves of DNA and RNA, it is likely that NPs can bind and occupy these sites (Fig. 3C). Computationally, it has been predicted that fullerene C_{60} preferentially binds to the minor groove of DNA [14] and major groove of RNA [66]. Atomistic molecular dynamics simulations performed using double-stranded (DS), single-stranded (SS) and damaged DNA indicated that C_{60} binds and deforms SS-DNA and then stably occupies damaged sites on DNA

[14,66]. Similarly, SWCNTs, upon their binding, were also shown to induce conformational changes in the DNA structure [61,62,67]. Another study showed that the interaction of aggregated hydrophobic polymers coated NPs with DS-DNA can induce unzipping, pulling the two strands apart [68]. Overall, these studies have suggested that irrespective of the negative charge on DNA, the van der Waals and hydrophobic interactions are the major driving forces involved in interactions of DNA with carbon based NPs (Fig. 3C). In addition to uncharged carbonaceous NPs, cationic NPs have also been extensively studied for their potential biomedical applications. Because DNA and RNA molecules are negatively charged, the cationic NPs – for example Au-NPs – could electrostatically interact with these macromolecules and affect their functions. A recent molecular dynamics simulation study reported that Au-NPs can bind and occupy both the major and minor groove of DNA [68]. Considering that DNA wraps around a cylindrical protein assembly to form chromatin with a diameter and height of 6 nm containing ~ 220 positive charges, the charge on the surface of NP may also determine its ability to interfere with the chromatin structure.

3. Selective interaction of biomolecules with nanoparticles

While comparable nano-dimensions of NPs and intracellular machinery make their interactions feasible, it is the compatibility of physico-chemical properties of nanosurfaces with those of biological objects that define the driving forces, specificity, consequences and outcomes of their interactions. Adsorption of biomolecules frequently modifies the NP surface. Consequently, interactions of NPs with proteins/lipids can enhance their biocompatibility [19,69] or enable the protein-modified NPs to be nontoxic or less toxic than the pristine NPs [70].

3.1. Adsorption of biomolecules on the surface of NPs affects their uptake and clearance

SWCNTs in their pristine state have a tendency to form bundle-like structures, due to strong hydrophobic interactions between them.

Similarly, amphiphilic lipids as well as amphiphilic and hydrophobic sites on proteins may tend to preferentially bind such NPs. The adsorption of biomolecules onto the surface of NPs is shown to affect their 3D structure and function, either by unfolding or by interfering with their stability and conformation [71–76]. Using molecular dynamics simulations, several groups have investigated and compared the adsorption of proteins onto the surface of different NPs, including graphene, C₆₀ and SWCNTs [77–79] and other nanomaterials [74]. These studies demonstrated that π – π stacking interactions of NPs with aromatic residues in proteins as well as hydrophobic interactions between proteins and NPs play a dominant role in driving such interactions [34,75,76,80], albeit the contribution of each interaction differed among different NPs investigated [78]. Studies by Hung et al. [74] indicate that, in addition to π – π stacking interactions, positively charged amino acids – such as lysine and arginine – can play a role in the adsorption of proteins onto the surface of nanoparticles. Importantly, computational studies by several groups indicated that the interactions between proteins and NPs can be affected by the shape and chemical composition of the NPs, as well as the structure and sequence of the proteins [71,74,78,81]. Similarly, molecular docking studies demonstrated that phospholipids can form an uninterrupted “coating” on the surface of SWCNTs, whereby the hydrophobic alkyl chains of the phospholipids were adsorbed onto the SWCNT, with their polar head groups pointing towards the aqueous phase [82]. These interactions of SWCNTs with proteins/lipids can result in a coating on NPs surface, ultimately masking NPs inherent properties. Moreover, proteins/lipid coating of the NPs surface can markedly affect the biological responses triggered by interactions with a variety of cellular receptors and leading to the modified uptake, toxicity and distribution of NPs [83,84]. An excellent recent review by Monopoli et al. [85] summarized the basic concepts of NP corona and highlighted major pathways through which the protein/lipid corona on the NP surface may affect the biological outcomes. For example upon pulmonary exposure, the presence of either surfactant lipids or protein coating on the surface of NPs markedly enhanced their uptake by phagocytosing cells [78,82,86]. It is possible that this effect is due, at least in part, to the recognition of specific classes of lipids by receptors similar to those involved in the uptake of apoptotic cells by macrophages [87]. Interestingly, NPs pre-coated with surfactant may be less susceptible to coating by other abundant proteins, like albumin, resulting in modified pro-inflammatory responses of macrophages elicited by the exposure to non-coated NPs [83].

3.2. Interactions leading to biodegradation of NPs

Recent studies on oxidative enzymatic biodegradation of carbonaceous NPs by myeloperoxidase (MPO) and eosinophil peroxidase (EPO) of inflammatory cells emphasized the importance of understanding the molecular details of interactions of biodegrading proteins with nano-surfaces [88]. By employing computational modeling studies, it has been demonstrated that interactions of basic/positively charged amino acids of the enzymes with the carboxyl moieties on the SWCNTs – positioning the NP in close proximity to the catalytic site of the enzyme – were essential for the effective catalysis [88–91]. To maintain activity, such selective interactions of nanoparticles must not interfere with the structural and functional integrity of the hemo-proteins (for ex: peroxidases). In contrast, small molecular weight oxidants such as hypochlorous acid may randomly attack SWCNT at multiple sites independently of the presence of negatively-charged groups on their surface. The combined action of hypochlorous acid and MPO reactive intermediates provides for the effective biodegradation of both pristine and carboxylated SWCNT.

4. Conclusions

Broad applications of nanotechnology in biomedicine depend on profound understanding of mechanisms and pathways through

which nanomaterials can affect biological systems. In the next decade, it will be important to elucidate how the physicochemical properties of nanomaterials and their by-products interact with subcellular organelles, cells, tissues, and organisms. Understanding the molecular details that underlie the interactions of NPs with nano-machinery of cells can provide insights and facilitate design and engineering of new generations of NPs with minimized toxicity. In this review, we described several major mechanisms through which properties of NPs – size, shape, chemical composition, and surface characteristics – can affect the nano-sized intracellular targets. Because long term effects of NPs are difficult to experimentally reproduce in cells or *in vivo*, structure based computational modeling represents a useful and effective approach to predict possible toxicological effects. It also provides a platform for designing and conducting meaningful experiments *in vitro* and *in vivo*. In addition, computational and structural modeling approaches can be used to identify important patterns in the nanoparticle-biomolecule interactions that can lead to the development of novel strategies and tools to further the field of nanotechnology. An interesting example is a recent study by Calvaresi et al. [92] demonstrating that hydrophobic pockets in proteins and enzymes can be used as tools for sorting and separating nanoparticles of different sizes, shapes and chiralities. Finally, by providing molecular insights into the possible mechanism of interactions of nanoparticles and with nano-machinery of cells, computer modeling emerges as a powerful tool in risk assessment studies.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

Abbreviations

NPs	nanoparticles
CNT	carbon nanotubes
SWCNT	single wall carbon nanotubes
MWCNT	multi-walled carbon nanotubes
EPO	eosinophil peroxidase
MPO	myeloperoxidase
MTs	microtubules
VDAC	voltage dependent anion channel
RNA	ribonucleic acid
DNA	deoxyribonucleic acid
HIV	human immune deficiency virus
HIV-1P	HIV-1 protease
KcsA, Kv1.2 and MthK	prokaryotic, voltage dependent, and ligand gated potassium channels, respectively
K ⁺	potassium ion

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