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Exploring the intricacies of protein–nanoparticle interaction and its implications in chronic diseases: a comprehensive review

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The protein and nanoparticle interaction is the basis of nanoparticle bio-reactivity. Nanoparticles upon interaction with proteins form a protein corona, altering their characteristics. This corona influences nanoparticles' biodistribution, pharmacokinetics, and therapeutic efficacy. The complex protein–nanoparticle interactions have a significant impact on the emergence of chronic inflammation and chronic diseases. This study is a comprehensive review that explores the dynamic nature of protein–nanoparticle interactions, emphasizing their long-term effects on sustained inflammatory responses and subsequent implications for various chronic conditions, and not an exhaustive review of all aspects. This study investigates the role of nanoparticle characteristics such as the size, shape, and surface charge in the formation of a protein corona, addressing the molecular aspects and cellular pathways involved. The connection between protein–nanoparticle interactions and chronic inflammation is deeply explored in the context of specific diseases, including cardiovascular disorders, neurological conditions, respiratory ailments, metabolic disorders, autoimmune conditions, and cancer. Insights from *in vivo* and clinical studies, coupled with discussions on genotoxicity, immunotoxicity, and mitigation strategies, contribute to a deeper understanding of the broader implications of these interactions. Nevertheless, this serves as a foundational framework for grasping the pivotal advancements and breakthroughs achieved *via* recent novel perspectives concerning the advanced methodologies for investigating protein–nanoparticle interaction and its correlation with chronic diseases. Additionally, this endeavour seeks to identify existing knowledge gaps demanding thorough exploration and offers insights for enhancing our knowledge of the interplay between protein–nanoparticle interactions and chronic disease pathogenesis. By addressing ethical considerations and public perceptions, this review outlines future research directions, highlighting the importance of extending our understanding of the safe and effective integration of nanotechnology into a broad range of applications.

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1. Introduction

Nanoparticles are extremely tiny particles, typically with the size of one to one hundred nanometres. These small-sized particles, differing in physical and chemical characteristics, are increasingly proven to be inevitable in various applications including consumer products as well as healthcare devices. It is therefore important that their increased presence within our surroundings instigates the need to understand how these tiny particles interact with biological systems specifically proteins. The dynamic interaction of proteins with nanoparticles gives rise to a protein corona that dictates several biological processes.[1] The process of the complex formation of the protein corona on the surface of the nanoparticle is, therefore, an important aspect of nanotoxicology. When nanoparticles come into contact with biological fluids, a dynamic process is activated, which determines the biological fate of these particles.[2] The size, shape, surface charge, and composition of the nanoparticles govern or regulate both the stability and structure of the protein corona. This further changes the chemical and physical characteristics of nanoparticles in such a way that they may have different physiological functions in living organisms.[3] Knowing the intricacies or complexities of the biological processes would, therefore, demand the knowledge of the long-term implications of the interplay between proteins and nanoparticles. The fact that cells recognize and take up the protein–nanoparticle

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complexes reveals a rather complicated web of interactions within life.[4] Furthermore, the biodistribution in the body may have immense implications for how they end up in accumulating or aggregating together in a variety of tissues apart from significantly influencing their interactions. These interactions take place at a nanoscale where conventional biological responses may appear; hence, there is a need to have a keen understanding of these complex phenomena to determine the biological fate of nanoparticles and their possible consequences over time. In particular, it examines how such interactions can have an impact on chronic health conditions and persistent inflammation. Such a finding has major implications since it may completely alter our understanding regarding the toxicology as well as green integration of nanomaterials into various applications. This review focuses on chronic inflammation, a condition also referred to as long-lasting immune response. Often chronic inflammation is one of the major causes of many chronic diseases, including metabolic syndromes, respiratory diseases, neurological complications, and cardiovascular disorders.[5] Linking the dots of chronic inflammation to protein–nanoparticle interaction could be used to explain how nanomaterial exposure can lead to or worsen persistent medical conditions. Perhaps nanoparticles could play an important role in their pathophysiology because this will help elaborate on the cause of these diseases and their

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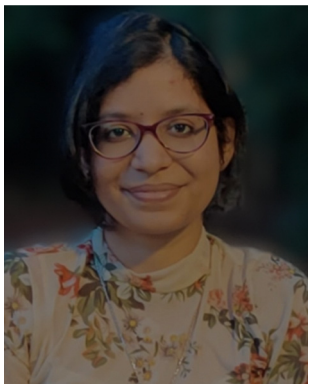

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Table 1 Effect of nanoparticle properties on protein adsorption and cellular uptake

Nanoparticle property	Effect on protein adsorption	Effect on cellular uptake
Size (small: <50 nm, large: >100 nm)	Smaller NPs adsorb fewer but more specific proteins. Larger NPs adsorb a higher amount of proteins, forming a dense corona	Small NPs enter cells *via* endocytosis more efficiently. Large NPs may be recognized and cleared by macrophages
Surface charge (positive *vs.* negative)	Positively charged NPs strongly adsorb negatively charged plasma proteins (*e.g.*, albumin). Negatively charged NPs adsorb opsonins, leading to immune recognition	Positive NPs show higher uptake due to electrostatic interactions with negatively charged cell membranes. Negative NPs may have lower uptake but higher circulation time
Shape (spherical, rod-like, irregular)	Spherical NPs form a uniform protein corona. Rod-like NPs show anisotropic protein adsorption, altering their biological interactions	Rod-like NPs exhibit higher uptake by certain cells but slower clearance. Irregularly shaped NPs may be recognized by the immune system
Surface coating (PEGylation, proteins, and lipids)	PEGylated NPs resist protein adsorption (stealth effect). Lipid-coated NPs may mimic cell membranes, altering protein interactions	PEGylation enhances circulation time by avoiding immune recognition. Protein-functionalized NPs may show selective uptake by target cells

widespread public health consequences. Protein–nanoparticle interaction has come a long way over the years, influencing nanomedicine development for chronic illnesses. Research conducted in the early 2000s identified the "protein corona" formation, which determines nanoparticle behavior within biological systems.[6] The 2010s saw the functionalization of nanoparticles as great agents for targeted drug delivery and imaging in disease conditions such as cancer and diabetes. Mid-decade investigations more extensively probed their function of regulating immune response, giving way to new concepts in immunotherapies. The understanding of these links between proteins, nanoparticles, chronic inflammation, and chronic diseases may facilitate the development of safer nanomaterials and improve risk evaluation procedures. In this respect, this paper adds to the active debates on the safe integration of nanomaterials into our fast-changing technological world by discussing complex interactions that occur between proteins and nanoparticles, which have effects on chronic inflammation and diseases associated with them. It provided an in-depth focus on key issues and discoveries regarding protein–nanoparticle interactions. The goal of this study is to identify current knowledge gaps that must be filled in order to increase our understanding of the complex relationship between protein–nanoparticle interactions and the underlying causes of chronic diseases.

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2. Nanoparticle characteristics

2.1. Impact of the nanoparticle size on the nature of protein corona

A number of studies have indicated that the size of nanoparticles shapes the protein corona synthesis, implying that it is an important determinant or factor for nanoparticle interactions and their ultimate biological fate. A protein corona is formed whenever nanomaterials come into contact with biological fluids because it causes proteins in the surrounding environment to adsorb on its surface.[7] Various experiments have shown that smaller nanoparticles usually have a higher area to volume ratio when compared to larger ones. Thus, a more diverse or heterogeneous corona was formed, and proteins are absorbed. For example, Bewersdorff *et al.* studies demonstrated that smaller gold particles showed broader protein coronas and greater protein binding than larger particles.[8] Similarly, Ma *et al.* in a review article[9] also suggested that the state of the protein corona, such as their thickness, composition or quantity, affects several aspects of the gold nanoparticles' behaviour such as biodistribution, cytotoxicity, cellular uptake and cancer targeting (Table 1). The size-dependent protein corona could affect nanoparticles' biology and control their interaction with cells and tissues (Fig. 2). To adapt nanoparticles for a variety of uses, including for drug delivery, diagnostics, and nanotoxicology applications, it is crucial to understand the intricate nature of the interaction between the protein corona and nanoparticle size. In order to obtain a more comprehensive knowledge of the size-specific effects on protein corona dynamics, more research should be conducted to investigate these interactions in other nanoparticle types and biological environments (Table 2).

Table 2 Several types of nanoparticles along with their sizes, compositions, methods for surface modification, and challenges

Nanoparticle type	Size range (nm)	Composition of protein corona	Surface modification techniques	Challenges	Properties	Impact of protein corona	Ref.
Gold	5–100	Serum albumin, immunoglobulins, fibronectin	Ligand exchange, PEGylation, self-assembly	Stability in biological fluids, protein corona heterogeneity	Unique optical, electrical, and catalytic properties due to size, shape, and surface plasmon resonance (SPR).	Alters surface chemistry and charge. Reduces SPR effect, impacting imaging and sensing applications.	10
Silver	1–100	Serum albumin, transferrin, fibrinogen	Ligand exchange, surface modification	Toxicity concerns, interference with biological processes	Silver nanoparticles: known for antimicrobial properties.	Shields silver nanoparticle surface from direct interaction with bacterial membranes, mitigating antimicrobial effects.	11
Iron oxide	5–100	Serum albumin, fibrinogen, transferrin	Coating with surfactants, surface functionalization	Clearance by the reticuloendothelial system (RES), magnetic aggregation *in vivo*	Superparamagnetic properties used in MRI, hyperthermia treatment, targeted drug delivery	Affects magnetic properties and colloidal stability, alters magnetic responsiveness, impacting MRI performance, and influences biodistribution and cellular uptake, affecting therapeutic efficacy. Surface coatings reduce protein corona formation and prevent clearance by the reticuloendothelial system (RES)	12
Silica	10–200	Serum albumin, immunoglobulins, lysozyme	Salinization, coating with polymers	Aggregation in biological fluids, cytotoxicity	Tunable porosity, high surface area, ease of surface modification; ideal for drug delivery, gene therapy, and bioimaging	Dynamic protein corona affects dispersibility and pore accessibility. Dense protein corona may block pores, hindering drug release. Surface modifications (*e.g.*, PEGylation) minimize protein adsorption and improve stability in biological environments	13
Quantum dots	2–10	Serum albumin, transferrin, immunoglobulins	Ligand exchange, encapsulation with polymers	Cadmium toxicity, biocompatibility issues	Small size (2–10 nm), tunable optical properties, used in imaging, sensing, and optoelectronics	Protein corona alters fluorescence properties and quantum yield. Biocompatibility issues arise due to core materials like cadmium. Encapsulation strategies (*e.g.*, polymer coatings) mitigate toxicity and control protein adsorption	14
Liposomes	50–500	Serum albumin, immunoglobulins, lipoproteins	Surface functionalization, encapsulation	Stability during storage, drug leakage	Spherical vesicles with lipid bilayer structure; used in drug delivery, vaccines, and cosmetics; biocompatible and biodegradable	Protein corona affects stability, immunogenicity, and drug release kinetics. Corona composition influences targeting efficiency and cellular uptake. Surface modifications (*e.g.*, PEGylation) reduce protein adsorption and prolong circulation time. Liposome–protein complexes may trigger immune responses or enhance therapeutic efficacy	15
Carbon nanotubes	1–100	Serum albumin, fibrinogen, immunoglobulins	Functionalization with polymers, covalent	Cytotoxicity, haemolytic activity, protein corona complexity	High aspect ratio, mechanical strength, thermal and electrical conductivity; widely explored for drug delivery, tissue engineering, and biosensing	Strong protein adsorption due to hydrophobic nature, forming a dense and heterogeneous protein corona; changes in dispersibility and cellular uptake; influences cytotoxicity and immunogenicity; may trigger	16

Table 2 (*continued*)

Nanoparticle type	Size range (nm)	Composition of protein corona	Surface modification techniques	Challenges	Properties	Impact of protein corona	Ref.
						immune responses or inflammatory reactions; functionalization (*e.g.*, PEGylation) reduces protein adsorption and improves biocompatibility	
Polymeric	10–500	Serum albumin, immunoglobulins, lysozyme	Encapsulation, surface functionalization	Stability issues, immunogenicity, drug release control	Versatile, can be tailored for controlled drug release, often used in drug delivery and therapeutics	Protein corona affects stability, immunogenicity, and drug release kinetics; surface functionalization helps optimize formulations but corona composition still depends on the biological environment	17
Magnetic nanoparticles	5–100	Serum albumin, transferrin, fibrinogen	Coating with surfactants, surface functionalization with polymers	Magnetic aggregation, biocompatibility concerns, clearance by the reticuloendothelial system (RES)	Superparamagnetic properties; used in MRI, hyperthermia treatment, and targeted drug delivery; surface can be functionalized for specific targeting	Protein corona alters magnetic properties and colloidal stability; impacts biodistribution, cellular uptake, and therapeutic efficacy; dense corona may reduce magnetic responsiveness, affecting MRI performance; coatings like PEGylation or surfactants minimize corona formation and improve biocompatibility	18
Titanium dioxide	5–100	Serum albumin, immunoglobulins, fibronectin	Surface modification with silanes	Phototoxicity, biocompatibility, and protein corona heterogeneity	Photocatalytic properties; widely used in sunscreens, self-cleaning surfaces, and environmental applications; can generate reactive oxygen species (ROS)	Protein corona affects phototoxicity and biocompatibility; heterogeneous corona formation complicates understanding of interactions; surface modification with silanes reduces protein adsorption and enhances safety; corona composition influences immune recognition and inflammatory responses	19
Quantum rods	5–50	Serum albumin, transferrin, and immunoglobulins	Ligand exchange and encapsulation	Toxicity concerns and aspect ratio-dependent toxicity	Anisotropic shape with tunable optical properties; used in imaging, sensing, and optoelectronics; high aspect ratio compared to quantum dots	Protein corona alters fluorescence properties and quantum yield; aspect ratio-dependent toxicity impacts biological interactions; encapsulation strategies mitigate toxicity and control protein adsorption; dynamic corona affects cellular uptake and biodistribution	20
Dendrimers	1–10	Serum albumin, fibrinogen, and lysozyme	Surface functionalization and encapsulation	Cytotoxicity, immunogenicity, and protein corona complexity	Highly branched, monodisperse structures; used in drug delivery, gene therapy, and diagnostics; surface can be tailored for specific functionalities	Protein corona impacts cytotoxicity and immunogenicity; surface functionalization (*e.g.*, PEGylation) minimizes protein adsorption; corona complexity depends on dendrimer generation and surface charge; positively charged dendrimers adsorb proteins more strongly, influencing biological fate	21

2.2. Characteristics of nanoparticles impacting the protein corona

Studying how the properties of nanoparticles influence the development of protein coronas has provided significant knowledge of the complex interplay between nanoparticles and the biological environment. The impact of nanoparticle shape on protein corona dynamics has been shown by the studies such as a study conducted by Bewersdorff *et al.*,[22] who mentioned that nanocages might offer enhanced biocompatibility in comparision with the other shapes because of the highly curved areas and dense ligation on the flat surfaces due to which opsonisation may be reduced, leading to slower clearance by the immune system. The unique aspect ratio and surface curvature of each shape (as shown in Fig. 1) contributed to variations in protein adsorption influencing subsequent biological responses. The impact of nanoparticle shape on protein corona formation is less investigated. Nanoparticles with high curvature areas like nanocages or nanorods exhibit dissimilar protein adsorption profiles compared to spherical nanoparticles. High curvature prevents opsonization and immune recognition, while flat surfaces enhance dense ligation, suppressing nonspecific adsorption of proteins. Anisotropic shapes like nanorods impact protein adsorption according to their aspect ratio and surface orientation. Spherical nanoparticles, for instance, adsorb as homogenous coronas but are susceptible to nonspecific adsorption, whereas nanocages and porous architectures adsorb as heterogeneous coronas due to their intricate geometries.[23] Filamentous geometries such as carbon nanotubes promote strong protein adsorption on the basis of hydrophobic surfaces. Biological effects of shape–corona interactions include alterations in cellular uptake, circulation time, and targeting efficacy. Regarding surface charge, investigations done by Bewersdroff *et al.*[22] highlighted that the surface charge on dendritic polyglycerol-coated AuNPs plays a crucial role with regard to the proteins present in the corona. In contrast to their negatively charged or neutral counterparts, positively charged nanoparticles showed unique corona characteristics, highlighting the importance of electrostatic interactions in forming the protein corona.[23] Surface charge also plays a central role in the formation of protein corona, since it governs electrostatic interactions between nanoparticles and proteins. Yet, methods of regulating surface charge with precision, especially by techniques like self-assembled monolayers (SAMs), have not been extensively discussed. SAMs are organic compounds that automatically come together in a very ordered monolayer on solid surfaces in order to provide control over surface nanoparticle properties with high precision.[24] For example, thiol-based SAMs are used with metal nanoparticles like gold, and thiols containing carboxyl, amine, or hydroxyl functional groups are chosen to design surface charge. In the same way, silane-based SAMs are used with oxide nanoparticles like silica or titanium dioxide. Positively charged nanoparticles adsorb negatively charged proteins, while negatively charged nanoparticles repel them, and neutral or zwitterionic surfaces inhibit nonspecific adsorption. The surface charge effect is also utilized in determining protein affinity, corona structure, and biological response, and hence it is a very significant consideration in designing biocompatible nanoparticles.[25] Target drug delivery, reduced immunogenicity, and controlled release are some of the strategic applications of SAMs. There are limitations, however, like SAM degradation under physiological conditions and the necessity for standardization protocols. In addition, Poulsen's[26] research brought attention to the vital and dynamic role that nanoparticle composition, including surface coatings and materials, plays in regulating the characteristics of protein coronas. According to these investigations, when the total amount of corona in lung fluid was lower than that in foetal bovine serum and bovine serum albumin coronas, there was a signal of an inflammatory response due to increased production of TNF-α, MIP-2 (macrophage inflammatory protein 2), and interleukin 6 (IL-6). When all factors are considered, these results demonstrate how complex protein corona formation is and how it depends on the size, composition and surface charge of nanoparticles. This provides crucial information for rational development of nanomaterials for a variety of applications.

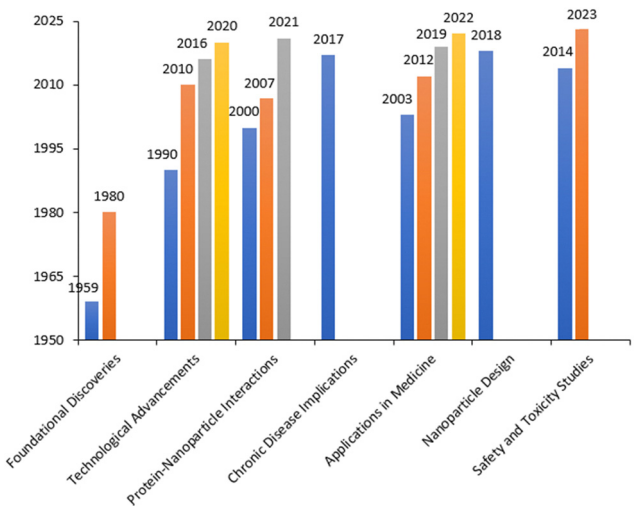

Fig. 1 Timeline of key developments in protein–nanoparticle interactions and their implications in chronic diseases.

2.3. Effects of surface modification on protein–nanoparticle interactions

Surface modification techniques play a critical role in tailoring or designing the interaction between proteins and nanoparticles, thereby influencing various biological applications. A study conducted by Lynch and Dawson[27] investigated the effects of surface modifications on protein–nanoparticle interactions. When nanoparticles are coated with biocompatible polymers, such as polyethylene glycol (PEG), reduced protein adsorption has been observed, minimizing protein corona formation and enhancing colloidal stability.[28] When nanoparticles are PEGylated with biocompatible polymers such as polyethylene glycol (PEG), protein adsorption is minimized, inhibiting protein corona formation and favoring colloidal stability. PEG coatings form a hydrophilic, steric barrier on

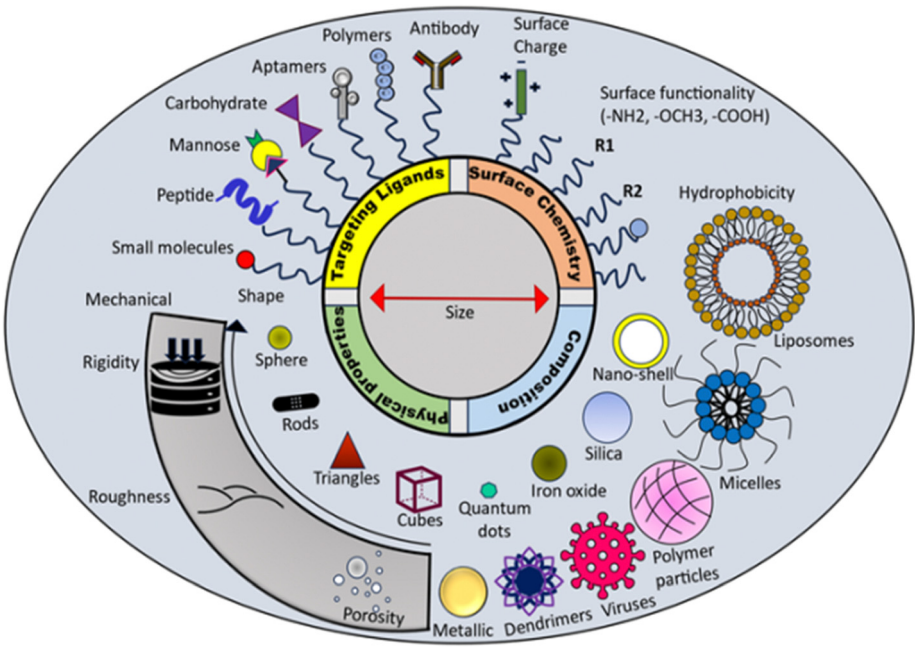

Fig. 2 Design of nanoparticles based on their physical properties, composition, and surface chemistry and their functionalization with a wide variety of ligands for biological targeting.

the nanoparticle surface, preventing proteins from accessing the core due to steric hindrance and decreased hydrophobic interactions.[29] This ''antibiofouling'' prevents the adsorption of nonspecific proteins, increasing biocompatibility and the circulation lifetime within the organism. On the other hand, efficiency of PEGylation is affected by parameters such as molecular weight, coating density, and nature of attachment. PEGylation will decrease the protein adsorption, but high-affinity-specific proteins continue to adsorb and form a selective corona that is capable of modulating nanoparticle behavior. Optimization of the PEGylation conditions is required to enhance optimized minimized biofouling as well as advantageous functional qualities of selective protein binding for therapy.[30] A similarly work conducted by Marruecos *et al.*[31] has studied the impact of surface charge modification on protein binding, clarifying how alterations in charge influence the conformation and composition of the adsorbed proteins. Furthermore, the study conducted by Yusuf and Casey[32] highlighted the importance of surface functionalization in mitigating nanoparticle-induced inflammation and also showcased the potential of surface modification techniques in enhancing the biocompatibility of nanoparticles for various applications in drug delivery and diagnostics. Surface modification approaches have shown enormous potential in enhancing the biocompatibility of nanoparticles for safe and efficient use in biomedical applications such as drug delivery, diagnostics, imaging, and regenerative medicine. Through the regulation of nanoparticle surface characteristics using methods like PEGylation, ligand conjugation, encapsulation, or biocompatible polymer coating, scientists can inhibit nonspecific protein adsorption, reduce immunogenicity, and extend circulation time in the biological system. The alterations not only enhance nanoparticle stability and targeting efficiency but also enable controlled drug release and specific binding with target cells or tissues. For instance, PEGylated liposomes, polymeric nanoparticles, and functionalized quantum dots have shown excellent success in reducing toxicity and improving therapeutic efficacy. Nevertheless, challenges like long-term retention of stability, prevention of coating degradation, and overcoming possible cytotoxicity or immune reactions persist. Despite these challenges, surface modification technologies have opened up the possibility of designing nanoparticles with improved efficacy and reduced side effects, a promising platform for next-generation nanomedicine and personalized medicine solutions.[33] Insights into the effects of diverse modification techniques have contributed to the development of nanoparticles with enhanced efficacy and reduced adverse effects in different biomedical applications.

2.4. Strategies for protein corona formation on bare nanoparticle surfaces and surface protected nanoparticles

On bare nanoparticle surfaces, proteins from biological milieus such as blood plasma or serum adsorb rapidly due to the high surface energy of bare nanoparticles and lack of protective films. Physicochemical forces such as electrostatic interaction, hydrophobic forces, van der Waals forces, and hydrogen bonding are responsible for adsorption. The resultant protein corona is typically heterogeneous and consists of a ''hard corona'' of tightly bound proteins and a ''soft corona'' of weakly bound proteins that exchange dynamically with the surrounding environment. Naked nanoparticles are, however, plagued by problems such as uncontrolled adsorption producing unpredictable corona composition, enhanced immunogenicity by opsonization, cytotoxicity by denatured proteins, and reduced

Table 3 Comparison of bare and surface-protected nanoparticles

Aspect	Bare nanoparticles	Surface-protected nanoparticles
Protein adsorption	High and uncontrolled	Reduced and controlled
Biocompatibility	Low (high immunogenicity and cytotoxicity)	High (minimized immune response and toxicity)
Circulation time	Short (rapid clearance by RES)	Long (extended circulation due to reduced opsonization)
Corona composition	Heterogeneous and dynamic	Homogeneous and predictable
Applications	Short-term (imaging, diagnostics)	Long-term (drug delivery, theranostics)
Challenges	Unpredictable behavior, high toxicity	Complex synthesis, potential coating degradation

targeting efficiency.[34] Solutions to such problems are to precondition the nanoparticle surface with target proteins or peptides before their exposure to biological milieus and limiting their application to short-term uses such as imaging or diagnostics where their prolonged circulation is not required. Surface-protected nanoparticles, in contrast, are covered with biocompatible coatings such as polyethylene glycol (PEG), zwitterionic molecules, or other functional polymers that provide a physical layer to prevent protein adsorption.[35] These coatings restrict nonspecific protein binding by steric hindrance yet enable controlled corona formation. Surface-protected nanoparticles have a variety of advantages, such as increased biocompatibility, increased circulation times in the blood by reduced clearance by the reticuloendothelial system (RES), improved targeting efficiency by coupled ligands, and increased reproducibility of corona composition. This notwithstanding, challenges such as potential coating degradation with time, exposing the bare surface, and the complexity and cost of synthesizing functionalized nanoparticles remain. Optimal surface protection involves sophisticated methods such as layer-by-layer assembly, zwitterionic coatings, stealth polymer incorporation (*e.g.*, PEGylation), and targeted functionalization to optimize stability, biocompatibility, and therapeutic efficacy (Table 3).[36]

2.5. Findings and challenges

Past research studies have thoroughly examined the features of nanoparticles, presenting size-dependent properties, surface effects, thermal behaviour, quantum phenomena and interactions with biological systems (Table 2). Zhang *et al.*[37] in their study have shown the size-dependent nature of nanoparticles' properties, highlighting how their optical and catalytic behaviour changes as the size decreases. Guo *et al.*[38] further studied the effects of surface modifications on the physicochemical properties of iron oxide nanoparticles and their performance as anticancer drug carriers. Adekoya *et al.*[39] elucidated the effects of quantum confinement and shape on the band gap of core/shell quantum dots and nanowires. The distinct interactions between nanoparticles and biological systems, paving the way for applications in drug delivery and imaging, have been studied by Aibani *et al.*[40,41] Despite these advancements, challenges persist in the synthesis, characterization, toxicity, stability and scale-up of nanoparticle technologies.

Challenges and difficulties have been faced in achieving uniformity and reproducibility in nanoparticle synthesis on a large scale.[42] Accurate characterization techniques at the nanoscale and the potential toxicity of nanoparticles, emphasizing the significance of understanding their biocompatibility, are needed.[43,44] Moreover, Rashidi *et al.*[45] have discussed the challenges in transitioning nanoparticle based technologies to large scale production (Table 4). Addressing the challenges is necessary for understanding the full potential of nanoparticles in various applications. The dynamic nature of protein corona formation over time complicates its characterization and prediction, necessitating advanced analytical techniques and computational modelling approaches for a deeper understanding. Future perspectives require the development of advanced analytical techniques such as mass spectrometry-based proteomics and computational modelling to provide detailed insights and prediction of protein coronas based on nanoparticle characteristics. Standardization of protocols and collaboration across disciplines will be crucial for advancing our collective understanding and translating research findings into practical applications, ultimately leading to the development of safer and more effective nanoparticle-based technologies.

3. Protein corona formation

3.1. Dynamic nature of the protein corona

The protein corona, which is formed upon the interaction of nanoparticles with biological fluids, exhibits a dynamic and evolving nature.[46] The protein corona composition is not static; it changes over time, which is influenced by the surrounding biological milieu and the physicochemical properties of nanoparticles.[47] It is formed rapidly upon exposure to biological fluids, and its composition evolves upon interaction of proteins with different affinities and dissociation from the nanoparticle surface.[7] This dynamic process is affected by factors such as nanoparticle size, surface charge and shape. First, proteins of high mobility and binding affinity, termed as ''fast binders'' or ''pioneering proteins,'' quickly adsorb on the surface of nanoparticles in seconds to minutes. These strongly adsorbed proteins with comparatively long-term stability form the ''hard corona'' core. Subsequently, low-affinity proteins or proteins with poor diffusion rates exchange dynamically with already adsorbed proteins, leading to dynamic changes in corona composition. This process, through the regulation of protein concentration, nanoparticle surface characteristics, and environmental conditions, achieves a pseudo-steady state in minutes to hours. As time increases, the ''soft corona'' made up of loosely adsorbed proteins is under constant replacement and dissociation by competitive binding of proteins of varying

Table 4 Summary of findings from selected studies showing size-dependent properties

Objective type	Methods used	Findings	Challenges	Ref.
To study the size-dependent catalytic properties of gold (Au) clusters	Synthesis of Au clusters, single molecule nanocatalysis, and/or IQ based detection	Variations in catalytic activity based on the size of individual (Au) clusters observed at the level of single clusters, showing a potent size-dependent impact on the catalytic characteristics of Au clusters in the formation and dissociation process, quantum size effect on the catalysis of individual clusters	Understanding the size-dependent properties and uncovering the unique size effect are the main challenges.	Zhang *et al.*[37]
To investigate the physicochemical properties of iron oxide nanoparticles with different surface modifications	A modified chemical co-precipitation method, surface modification techniques, doxorubicin (DOX) was used as a model drug	Surface modifications were found to affect the physicochemical properties of iron oxide NPs The surface coatings affected the crystalline structure of IONPs where magnetization decreases with an increase in the amount of their organic coatings	Understanding how surface modifications affect the physicochemical properties Investigating the drug loading capacities, drug release patterns, and the impact of surface modifications Assessing the colloidal stability of the surface-modified IONPs Evaluating the cytotoxicity of the surface modified IONPs Ensuring the biocompatibility and stability of the surface modified IONPs under physiological conditions is a key challenge	Guo *et al.*[38]
To identify the similarities and differences between these colloidal nanoscale materials (nanoparticles (NP), nanocrystals (NC), and quantum dots (QD)) by providing the correct semantics for the discussion of the salient processes	State-resolved pump/probe approach, examination of carrier cooling processes, analysis of linear absorption and photoluminescence spectra	Clarification of terminology Historical perspective, quantum confinement effects, function across different families of nanoparticles	Faces challenges in describing the intermediary regime of quantum dots (QD) and understanding the differences between a nanocrystal (NC), which may be bulk-like and a QD, which interpolates between the bulk and the molecular limits. The dynamics problem in QD is also a challenge	Kambhampati *et al.*[39]
To study the thermal properties	Characterization techniques (SEM, EDS, FTIR, and DSC), COMSOL MULTIPHYSICS 5.2a based numerical simulation, uncertainty analysis	Introduced a novel highly stable FS-PCM showing improved thermal properties	Miniaturization of electronics	Raj *et al.*[40]
To improve the energy storage characteristics of FS-PCMS in heat sink applications		A decrease in average heat sink temperature, validating the effectiveness of the phase change material	Environmental factors	
To validate the experimental results numerically using COMSOL software		Addition of MWCNTs and GNP nanoparticles significantly increased the thermal conductivity of the FS-PCM	Heat dissipation challenges posed by advanced electronic devices	
Investigate how chitosan interacts with cell membranes Explore the *in vivo* distribution of chitosan nanoparticles and their bioavailability Discuss the toxicity of chitosan formulations and their implications Address the challenges and potential of chitosan formulations, examine the role of chitosan nanoparticles in advanced drug delivery systems.	Surface modification technique, covalent modifications, clinical investigations, exocytosis techniques	Chitosan showed pH-dependent detachment of cells, enhanced cell adhesion observed with specific modifications, chronic lung congestion found in high-dose groups, minimal toxicity reported in most studies, biodegradability of chitosan influenced by various factors, chitosan's unique properties make it a promising choice for nanoparticulate drug delivery	Challenges in clinical translation due to unforeseeable issues, standardization of extraction methods and analytical techniques, immunological activation and blood–brain barrier penetration is undesirable, regulatory approval and safety concerns, long-term toxicological studies	Aibani *et al.*[41]
To develop methods for large-scale synthesis of uniform-sized metal oxide nanoparticles, explore the potential medical applications of metal oxide nanoparticles, develop facile	Ball-milling, hydrothermal methods, coprecipitation methods, synthesis approaches for nanoparticles, surface modification techniques	Successful large-scale synthesis of uniform-sized metal oxide nanoparticles achieved, metal oxide nanoparticles show promise in medical applications, titania nanoparticles enhance drug delivery efficacy against	Large-scale production, toxicity concerns, blood–brain barrier crossing	Kwon *et al.*[42]

Table 4 (*continued*)

Objective type	Methods used	Findings	Challenges	Ref.
and economic ways to produce high-quality water-dispersible nanoparticles		cancer cells, biocompatibility and therapeutic potential achieved		
To provide a comprehensive review of various techniques used for the characterization of nanoparticles (NPS), to identify valuable techniques that merit further technical improvements	Transmission electron microscopy (TEM), atomic force microscopy (AFM), scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS)	The wide use of NMR, FTIR, and SERS techniques The analysis of surface and frustration evidence, providing microscopic information on the internal magnetic order of the particles	The accuracy and resolution of many techniques	Mourdikoudis *et al.*[43]
Investigate factors influencing pharmacokinetics, develop multimodal interventions, explore integration of therapeutic and imaging agents, conduct long-term toxicity studies, investigate novel drug-loading techniques	Animal models, 3D cell culture model, behavioral tests, protein analysis, surface modification techniques, innovative electrochemical detection methods	Neuroprotective effects Anti-inflammatory and oxidative stress reduction Memory retention enhancement Stimulation of neurogenesis Mitochondrial protection Modulation of signaling pathways, selective cellular uptake	Complex design and optimization Drug delivery optimization Novel drug-loading technique development, understanding complex interactions, tailoring AuNPs for specific disorders	Chiang *et al.*[44]
Investigate toxicity and safety, assess efficacy and reproducibility Address regulatory challenges Optimize drug loading and release Enhance clinical translation	Experimental studies, clinical trial analysis, regulatory compliance assessment, drug loading optimization	Nanoparticles enhance bioavailability at tumor sites, nanoparticles help in early detection and improve accuracy and speed of diagnosis, updates on clinical trials, nanoparticle strategies in cancer therapy are explored	Toxicity and safety concerns, efficacy and reproducibility testing, regulatory compliance hurdles, drug loading and release optimization, clinical translation barriers, specificity, and sensitivity enhancement	Rashidi *et al.*[45]

affinities. The kinetics of corona formation is also dictated by the size, shape, surface charge, and hydrophobicity of the nanoparticles, influencing the specificity and strength of protein binding.[48] Understanding all these kinetic processes in depth is important since the dynamic protein corona can significantly affect the behavior of the nanoparticle, such as cellular uptake, biodistribution, and therapeutic effect, and thus needs time-resolved quantification to capture the entire dynamic aspect of protein–nanoparticle interactions (Fig. 3). Likewise, a research study conducted by Wheeler *et al.* and Nandakumar *et al.* discovered the temporal evolution of the protein corona, explaining that the firstly formed corona complex undergoes significant changes in its protein composition in the initial few minutes to hours of exposure.[49,50] However, protein composition and quantity of the corona complex are able to modulate nanoparticle biological identity, followed by changes in cellular responses and interactions.[51] Zeng *et al.* highlighted the need for attention towards the dynamics of the protein corona in the study of the behaviors of nanoparticles in biological systems.[52] The researchers indicated that early protein adsorption influences the protein corona and it keeps on growing with time due to protein exchange. The basic understanding of these temporal changes is very important due to the interplay of the developing protein corona and the characteristics of nanoparticles such as, surface charge, size, and composition. It decides the ultimate fate of nanoparticles *in vivo*, which includes cellular uptake and biodistribution and probable immune-modulatory impacts. All of the above-mentioned studies provide insight into the dynamics of the protein corona, which can be potentially used to engineer and utilize nanoparticles for a variety of applications.

3.2. Factors influencing protein-adsorption

Mainly, the interactions of nanoparticles and protein adsorption onto the surface are very dynamic and complex events, which are therefore influenced by abundant interacting factors. Key elements include size, shape, and surface charge. Stronger protein adsorption is facilitated by smaller particles due to their larger surface area-to-volume ratios.[53] Surface chemistry has a significant impact on the substrate's physicochemical properties as well as the interactions between proteins and surfaces. Surface charge, hydrophobicity, and functional groups all have a significant influence on the adsorption behaviour, with electrostatic interactions particularly significant.[54] The shape and size of nanoparticles also have a significant impact on protein adsorption, as larger surface areas and unique geometries offer diverse binding sites and alter protein conformation upon adsorption.[23] Additionaly, surface charge whether positive or negative governs the affinity of proteins through electrostatic interactions, while hydrophobic surfaces facilitate adsorption through hydrophobic interactions[55] Protein conformational rearrangements upon adsorption onto the nanoparticle surface are a critical feature of protein–nanoparticle interaction, which often results in denaturation or reorganization of the protein structure with high consequential effects on biological response. Hydrophobic interactions predominate in adsorption onto hydrophobic

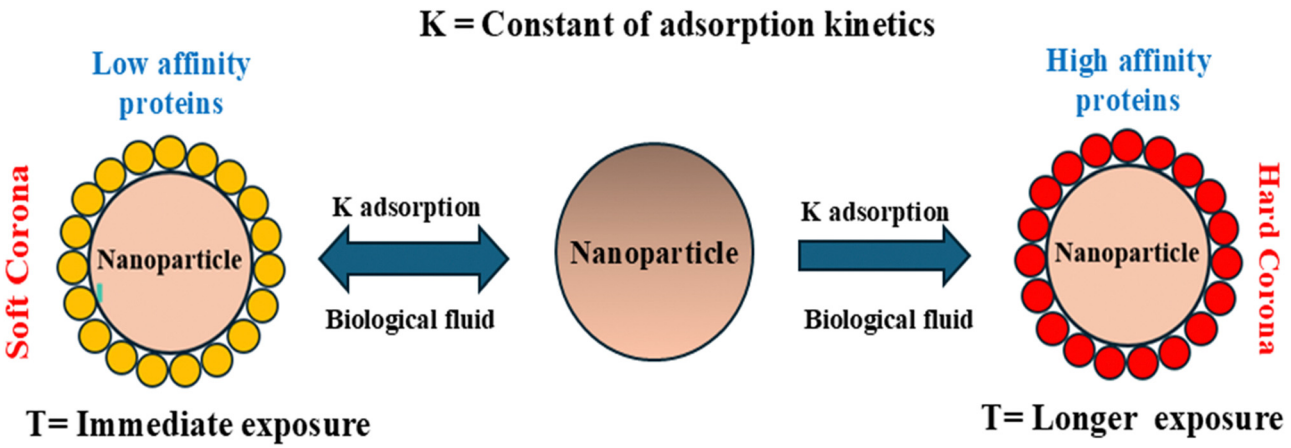

Fig. 3 Kinetic aspects of protein corona formation.

surfaces, resulting in surface reorientation of the hydrophobic faces of the protein and the hydrophilic faces away from the surface. This rearrangement can destabilize the native fold of the protein, resulting in partial or total denaturation. Denatured proteins can become inactivated, expose hidden epitopes, or form aggregates, triggering undesirable immune reactions or cytotoxicity. Adsorption on charged surfaces can also induce conformational rearrangements by electrostatic interactions, which can rearrange the protein secondary or tertiary structure. These rearrangements are highly surface-charge-, hydrophobicity-, and curvature dependent and thus require awareness of the correlation between surface properties and protein behavior.[56] Knowledge of such conformational dynamics is relevant to the design of nanoparticles with minimal denaturation effect and hence safe and effective for biomedical applications. Surface functionalization, such as introduction of polyethylene glycol (PEG) coatings, later modulated protein adsorption by altering its surface properties.[57] Moreover, the protein–surface interactions gets affected due to conditions like pH and ionic strength, by altering surface charge and protein conformation, thereby impacting the adsorption kinetics and the composition of the protein corona.[58] The intrinsic properties of the proteins themselves, including size, shape, surface charge and hydrophobicity, play a pivotal role in determining their adsorption behaviour and succeeding corona formation.[59] Protein concentration, exposure time and temperature are also critical factors, which influence the adsorption kinetics and corona composition. Additionally, in a biological environment, serum proteins, enzymes, and other biomolecules can competitively bind to the surface, altering the composition of protein corona and its biological interactions.[49] Understanding these complex factors is crucial for predicting and controlling protein adsorption and corona formation, which are crucial for designing nanomaterials for various biomedical applications, including drug delivery, imaging, and diagnostics.

3.3. Advanced methods for studying protein coronas

Advanced techniques or methods for examining and characterising protein coronas have become progressively sophisticated, reflecting the intricacy and relevance of this phenomenon in the field of nanomedicine, nanotoxicology, and nanobiotechnology (Table 5). Atomic force microscopy (AFM) and scanning electron microscopy (SEM) are the high-resolution imaging techniques that provide valuable insights into the size, morphology, and distribution of protein coronas on nanoparticle surfaces at nanoscale resolution.[60] Besides, modern spectroscopic techniques, such as surface enhanced Raman spectroscopy (SERS) and Fourier-transform infrared (FTIR) spectroscopy, provide precise information about the chemical composition, conformational changes, and intermolecular interactions within the protein corona.[61] Mass spectrometry-based methods, like liquid chromatography-mass spectrometry (LC-MS) and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), allow for the thorough profiling of the protein corona, identifying individual proteins and their post-translational modification.[62] The complexity and diversity of the corona composition could be revealed by mass spectrometry-based proteomic investigations, as conducted by Marichal *et al.*, which provide light on the specific or unique proteins adsorbed onto nanoparticles.[63] Furthermore, novel methodologies such as single-particle interferometric reflectance imaging sensing (SP-IRIS) provide real-time, label-free tracking of protein adsorption dynamics on single nanoparticles, illuminating details on the thermodynamics and kinetics of corona formation.[64] The protein corona is one entity that cannot be thoroughly understood without computational modelling and bioinformatics merged experimental approaches. They shed light on the structure–function relationship within the corona and also find out how proteins interact with nanoparticles.[65] In summary, these advanced methods permit a deeper understanding of the dynamics, composition, and biological relevance of the corona, therefore allowing for rational design of nanomaterials for diverse biomedical applications and also addressing safety concerns associated with nanotechnology.[66] There are new ways through which protein coronas can be studied in real time and complex biological environments.[67] Notably, more advanced imaging techniques like super resolution microscopy as well as fluorescence microscopy permit observation of protein coronas that exist on

Table 5 The most recent techniques for examining and characterising the protein corona, together with its benefits and drawbacks, and challenges

Methods	Application	Advantages	Disadvantages	Challenges	Barriers	Ref.
Mass spectrometry (LC-MS, MALDI-MS)	Identification and quantification of proteins	High sensitivity and specificity	Requires skilled personnel for operation	Identifying low abundance proteins	Expensive equipment and maintenance	72 and 73
		Quantitative analysis	Sample preparation can be time-consuming	Reproducibility of results	Access to specialized facilities	
Fluorescence spectroscopy	Real-time monitoring of protein interactions	Real-time measurement	Limited to fluorescently labelled proteins	Quantification of protein interactions	Signal interference from other molecules	74
		High sensitivity	May require modification of proteins	Detecting transient protein interactions	Limited to specific fluorescent labels	
Surface plasmon resonance (SPR)	Label-free detection of protein binding	Label-free detection	Limited to interactions on a sensor surface	Studying dynamic changes in protein coronas	Requirement of expensive SPR equipment	75
		Real-time measurement	Requires purified proteins for analysis	Reproducibility of experimental conditions	Specialized expertise in SPR operation	
Dynamic light scattering (DLS)	Analysis of size distribution of nanoparticles	Rapid and non-destructive	Limited to larger nanoparticles	Differentiating between free and bound proteins	Challenges with polydisperse samples	76
		Measures size distribution	Sensitive to aggregation	Quantifying protein adsorption onto nanoparticles	Requires suitable dispersants	
Cryo-electron microscopy (Cryo-EM)	Visualizing protein–nanoparticle interactions	Provides high-resolution images	Sample preparation can alter structures	Studying protein conformation changes	Access to expensive cryo-EM equipment	77
		Visualizes protein–nanoparticle interactions	Requires specialized expertise	Detecting transient or weak interactions	Sample stability during cryo-preparation	
Atomic force microscopy (AFM)	Characterization of protein–nanoparticle interactions	High-resolution imaging	Limited to surface analysis	Quantifying protein binding	Requires sample preparation	78
		Measurements in various environments		Determining protein corona thickness		
Scanning electron microscopy (SEM)	Visualization of protein–nanoparticle interactions	High-resolution imaging	Sample preparation may alter structures	Identifying protein distribution on nanoparticles	Limited to surface analysis	77
		3D topographic information		Determining nanoparticle morphology		
Surface-enhanced Raman spectroscopy (SERS)	Label-free detection of protein–nanoparticle interactions	High sensitivity	Enhancement limited to specific molecules	Detecting transient protein interactions	Requires optimized substrates	79
		Multiplex detection		Quantifying protein binding		
Fourier transform infrared spectroscopy (FTIR)	Characterization of protein–nanoparticle interactions	Provides structural information	Requires sample preparation	Determining protein secondary structure	Limited to surface analysis	74
		High sensitivity		Identifying protein–nanoparticle interactions		
Quartz crystal microbalance (QCM)	Real-time monitoring of protein adsorption	Label-free detection	Limited to surface-bound interactions	Quantifying protein binding	Requires specific surface functionalization	80
		Real-time measurement	Sensitive to changes in environmental conditions	Studying dynamic changes in protein coronas	Specialized equipment and expertise required	
Single-particle interferometric reflectance imaging spectroscopy (SP-IRIS)	Label-free detection and quantification of protein binding	Single-molecule sensitivity	High throughput	Analyzing heterogeneous samples	Requires specialized instrumentation and expertise	81

nanoparticles within live cells and tissues thereby giving insights into intracellular fate and biological interactions with particles.[68] The incredible level of control that microfluidic devices offer over experimental circumstances makes it possible to study protein adsorption kinetics and corona formation under physiologically relevant flow conditions.[69] It may also be noted that sophisticated biosensing technologies such as surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) provide sensitive and real-time protein adsorption monitoring onto nanoparticle surfaces, which could be used in characterizing corona formation kinetics and affinity constants.[70] Moreover, organ-on-a-chip models and three-dimensional tissue

culture systems offer more physiologically relevant conditions for assessing nanoparticle–protein interactions and thus give insights into how the protein corona affects cellular responses and tissue-level effects.[71] Understanding the dynamics, composition of the protein corona, biological implications can be achieved by utilizing advanced methods and novel platforms, thus leading to better comprehension of nanoparticle-based treatments, diagnostics, and nanotoxicity.

3.4. Implications for nanoparticle behaviour

One of the areas in nanotechnology that has gained much interest is the characterisation and studies on the protein corona, given that it affects the fate of nanoparticles within biological systems. The physicochemical properties, final destination, and biological interactions of nanoparticles are altered by a dynamic interface between the particles and their biological surroundings known as a protein corona.[82] First, proteins gets adsorbed onto its surface, which then will modify colloidal stability, aggregation propensity and cell uptake kinetics.[83] Protein adsorption on the plasma membranes most probably alters their excitation energy transfer rates.[84] Moreover, some soluble plasma membrane receptors can be sequestered into lipid rafts upon binding to cognate ligands or when expressed at high density in these domains.[51] Moreover, such a covering might prevent normal clearance by reticuloendothelial cells and promote opsonization *via* other mechanisms; prolonged circulation time was observed for particles with longer half-life.[85]

To add on, the protein corona manages the allocation of bioactive cargo molecules such as drugs or nucleic acids, which in turn affects their off-target consequences, therapeutic efficiency and release kinetics.[86] Importantly, nanoparticle features, biological fluid components and environmental circumstances impact both the dynamics and composition of the protein corona. For this reason, it is necessary to have a more comprehensive knowledge about how proteins interact with nanoparticles in different biological settings. This could help in curbing the potential dangers related to nanotoxicology and facilitate transfer of nanotechnology-based therapy and diagnostics into clinical practice by developing stronger nanomaterials for biomedical applications, optimizing drug delivery systems, and understanding better how the protein corona can influence nanoparticle behaviour.

4. Mechanism of interaction

4.1. Physicochemical interactions between nanoparticles and proteins

In the course of their interaction in biological systems, both proteins and nanoparticles are heavily influenced by a wide range of physical, chemical interactions that occur between them. At first, the protein corona is formed when proteins adhere to nanoparticle surfaces through electrostatic forces, hydrophobic forces and hydrogen bonding.[87] As a result, the charge, hydrophobicity and colloidal stability of nanoparticles are dynamically altered.[88] Furthermore, as the adsorbed proteins undergo structural changes on nanoparticle surfaces they affect their recognition by cellular receptors resulting in different biological responses.[89] The molecular mechanisms of protein–nanoparticle interaction are intricate and involve both thermodynamic and kinetic considerations that govern the formation and evolution of the protein corona. Thermodynamically, the interaction is governed by a delicate balance of forces, including electrostatic interactions, hydrophobicity, hydrogen bonding, and van der Waals forces, all of which determine the free energy change (ΔG) of the system. For instance, hydrophobic nanoparticles adsorb proteins tightly by hydrophobic interactions, while charged nanoparticles adsorb proteins of opposite charge, creating a stable corona. Kinetically, the process initiates with rapid adsorption of ''pioneer proteins'' on the nanoparticle surface, followed by dynamic exchange of proteins with proteins of varying affinities with time. Slowly diffusing or lower-affinity proteins exchange dynamically with the preadsorbed proteins, leading to the formation of corona composition. This dynamic process is regulated by protein concentration, nanoparticle size, nanoparticle surface charge, and environmental factors such as pH and temperature. Conformational changes of proteins upon adsorption are also crucial because these changes may lead to partial unfolding or denaturation, influencing the biological activity of the protein and recognition by cellular receptors. These changes may lead to exposure of cryptic epitopes, inducing immune responses or influencing cellular uptake.[90] These thermodynamic and kinetic considerations, binding affinities, and protein structural dynamics must be known to predict and control the biological consequences of protein–nanoparticle interactions (Fig. 4). In addition to this, the protein corona determines the pharmacokinetics and possibly the therapeutic efficiency of nanoparticles since it controls biodistribution and elimination from the body during *in vivo* studies.[91] Additionally, the protein corona composition and structure are affected by factors such as the surrounding biological milieu as well as surface chemistry, nanoparticle size and shape among others.[82] It is essential to understand these physicochemical interactions for fabrication of nanomaterials that will be used for biomedicine applications like administration of drugs or imaging diagnostics as well as risk assessment of nanotechnology.

4.2. Ligand–receptor interactions and their cellular implications

Ligand–receptor interactions are very important for intracellular signaling, communication, and regulation of biological processes. Examples of ligands include small molecules, proteins, and peptides; even nanoparticles can be ligands. They bind exactly to the cell surface receptors, hence starting a series of biochemical activities.[92] This may include changes in downstream signaling cascades, intracellular trafficking, and enhanced cellular uptake through endocytosis processes. The structure of the ligand and receptor binding sites is complementary, containing the right electrostatic attributes for these very selective interactions.[93] Upon contact with the

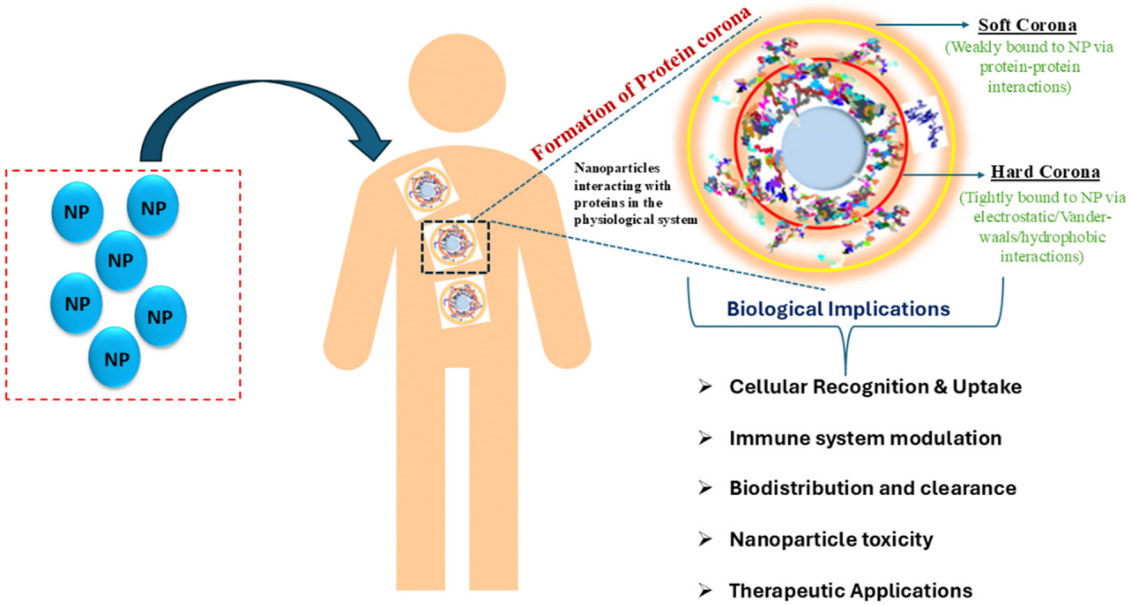

Fig. 4 Dynamic formation of the protein corona and its biological implications.

ligand, the binding can cause a change in the conformation of the receptor, changing gene expression, activating intracellular signaling pathways, and influencing cellular activity.[94] Processes like basic cell division, proliferation, and death and complex phenomena, such as immunological responses, synaptic transmission, and hormone regulation, have been influenced by these interactions.[95] Since the dysregulation of ligand–receptor interactions has been implicated in many diseases, including cancer, neurological conditions, and autoimmune diseases, this could represent a potential point of therapeutic intervention.[96] At the molecular level, a better understanding of the intricacies of ligand–receptor interactions is important for improving our understanding of cellular function and pathology, assisting us with the development of novel therapeutic strategies aimed at modulating these interactions for therapeutic benefit and further advancing applications in fields such as drug delivery and medical imaging.

4.3. Thermodynamics of protein–nanoparticle interaction

The thermodynamics of protein–nanoparticle interactions provide a basis for understanding the effects of nanoparticles on protein stability and aggregation. Interactions are goverened due to a delicate balance between electrostatic interactions, hydrogen bonding, van der Waals forces, and hydrophobic effects, all of which together influence the free energy change ΔG of the system. In the case of nanoparticles, surface properties will be importantly related to charge and functional groups. The interaction between ZnONPs and insulin fibrillation has recently been investigated in-depth, and it is established that the nanoparticle surface properties have a great influence on amyloidogenesis. ZnONPs possessing a negative surface potential are already reported to enhance insulin fibrillation, while those possessing a positive surface potential, ZnONPUnc, also did enhance fibrillation, with the reversed charge, further reinforcing that surface potential cannot alone explain the fibrillation behaviour. Notably, the ZnONP functionalized with amino acids like tyrosine and tryptophan can mitigate this effect by stabilizing insulin conformation through van der Waals interactions and hydrogen bonding with the key amyloidogenic sequences that reduce fibrillation and associated cytotoxicity. This underlines the potential of surface functionalization for nanoparticle-induced amyloid formation control with a view to ensuring high biocompatibility.[97] IAPP is also a 37-amino acid hormone involved in glucose regulation; however, because of pathological misfolding and aggregation into amyloid deposits in pancreatic β-cells, IAPP contributes to the development of TIIDM. In addition, these deposits themselves contribute to β-cell dysfunction and loss through the enhancement of oxidative stress, mitochondrial dysfunction, and apoptosis, exacerbating TIIDM. It should also be highlighted that IAPP is a functionally and structurally dynamic protein, importantly with gene expression, post-translational modifications, and associations with insulin and cellular membranes. Novel therapeutics measure action through stabilization of nonamyloidogenic structures of IAPP, inhibiting amyloid growth, fibril disruption, and targeting amyloid structures with antibodies, thus representing promising routes for reducing the injurious consequence of IAPP misfolding and improving treatments of TIIDM and associated amyloidopathies.[98] Consequently, by understanding and manipulating these interactions, researchers can develop nanoparticles that can decrease the adverse effects and improve biocompatibility, leading to improved therapeutic and diagnostic applications.

4.4. Cellular internalization pathways and downstream signalling cascades

Understanding the uptake pathways of chemicals in cells is a necessary condition for predicting the biological effect of nanoparticles. The route that nanoparticles take for entry into

the cells defines their fate inside the cells, their interaction with cellular factors, and subsequent biological responses.[99] A variety of internalization mechanisms, including caveolae-mediated endocytosis, clathrin-mediated endocytosis, and micropinocytosis, regulate the path that nanoparticles take into the cells.[100] Whether the nanoparticles remain inside the endosomes or are released into the cytoplasm and undergoes the lysosomal degradation pathway depends on the specific nature of these pathways.[101] Selection of the internalisation pathway influences not just the properties of the nanoparticles but also the cell responses. These cell responses have the capability to activate signalling cascades and change gene expression or have a general impact on cellular functions.[102] Therefore, in order to customise nanoparticle designs and forecast their biological effects thereby ensuring the development of safe and efficient nanomaterials for a wide range of applications in medicine and beyond a thorough understanding of cellular internalisation is essential.

5. Long term effects on chronic inflammation

5.1. Contribution of protein–nanoparticle interactions to sustained inflammation

Protein–nanoparticle interactions can have a substantial impact on sustained inflammation. In particular, nanoparticles are used for drug delivery, imaging, or diagnostics within the context of biomedical applications (Fig. 5). Upon being exposed to biological fluids, nanoparticles get coated with a dynamic layer of proteins, creating a protein corona.[46] This protein corona can change the surface characteristics of nanoparticles, impacting their recognition and absorption by immune cells. Certain nanoparticle–protein interactions may be identified by pattern recognition receptors (PRRs) on immune cells, such as Toll-like receptors (TLRs), resulting in the triggering of inflammatory pathways.[103] Furthermore, the protein corona may aid in the recognition of nanoparticles by phagocytic cells, such as macrophages, resulting in their engulfment and following activation.[47] After being taken up, nanoparticles can initiate intracellular signalling pathways that result in the creation and release of pro-inflammatory cytokines, *e.g.* tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6).[104] Additionally, nanoparticles might cause oxidative stress by producing reactive oxygen species (ROS), worsening the inflammatory response situation.[105] To further mitigate any negative effects associated with inflammation induced by nanoparticles, a thorough knowledge of the complex relationship between nanoparticles and the immune system is necessary for the production of safe and effective nanomedicines. Cytokine regulation is greatly influenced by protein–nanoparticle interactions that have shown considerable immunomodulatory effects within this biological environment.[106] When nanoparticles come into contact with biological fluid formation of protein corona takes place that is developed on the surface of nanoparticles, which further initiates a cascade of immunological reactions. Additionally, because of the long-term presence of the nanoparticles in the human system due to their prolonged circulation time or inefficient removal mechanism, this inflammatory

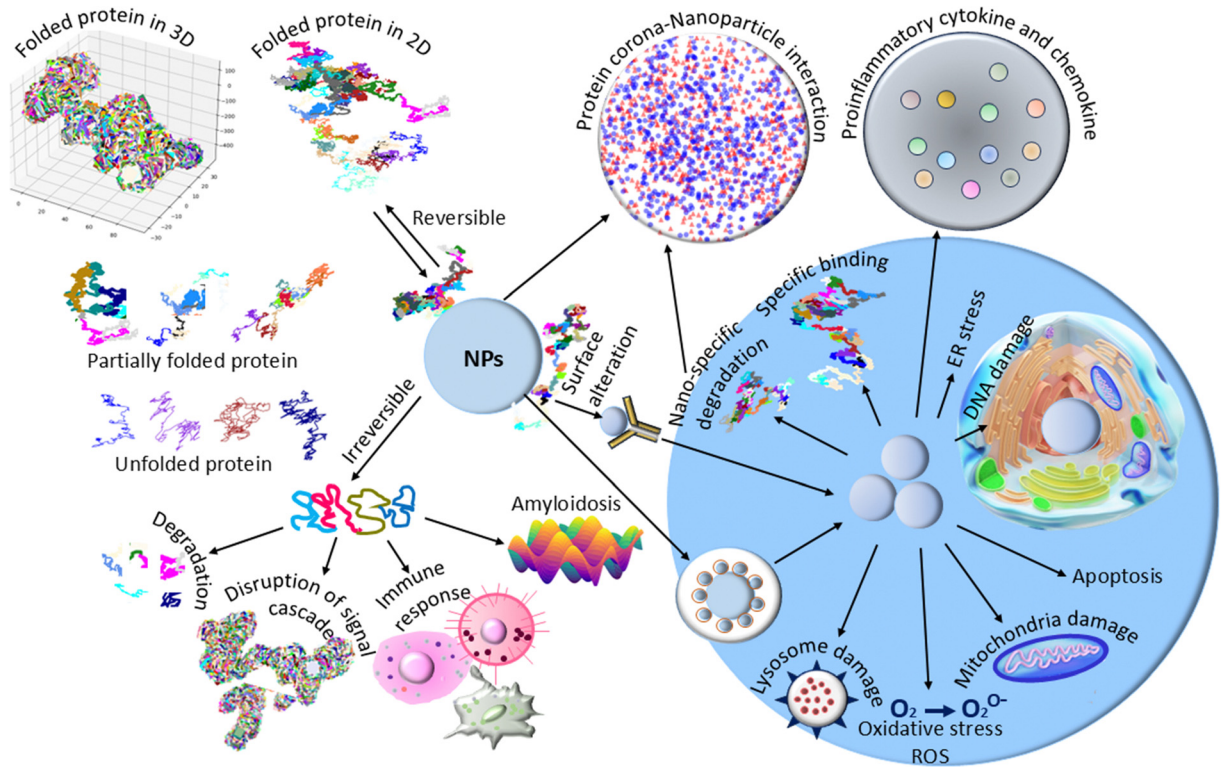

Fig. 5 Protein–nanoparticle interaction and its role in chronic inflammation.

response may persist to cause chronic inflammation and tissue injury.[107] The two cytokines that are released upon activation of immune cells and help in coordinating the inflammation are tumour necrosis factor-alpha and interleukins.[108] For the homeostasis of the immune system, these cytokines have to be strictly regulated, and the protein–nanoparticle interaction at a longer time may induce abnormal production of these cytokines, thereby contributing to chronic inflammatory disorders.[109] Understanding the complex relationship between cytokine regulation and nanoparticles holds the key to tailoring nanomaterials to achieve appropriate immunomodulatory profiles that can enhance the efficacy and safety of biomedical applications.

5.2. Linkages between chronic inflammation and chronic diseases

These complex interplays between chronic inflammation and long-term health problems are modulated by the interactions between proteins and nanoparticles.[110] Many medical diseases can develop and can be exacerbated by long-term exposure to these inflammatory inducers. For example, in the case of atherosclerosis (heart disease), inflammation transmitted by nanoparticles can contribute towards the problem.[111] These interactions between proteins and nanoparticles can drive chronic inflammation, which may worsen neurodegenerative diseases by enhancing neuronal damage.[112] Moreover, it is likely that the deregulation of immune responses promoted by nanoparticles contributes to the pathogenesis of autoimmune disorders.[113] The immunomodulatory mechanisms and molecular processes implicated in protein–nanoparticle interactions need to be elucidated to investigate the intricate connections present between a number of chronic ailments and chronic inflammation. In fact, a number of chronic illnesses such as cancer, neurological disease, and cardiovascular disease are noted to be chronic inflammatory conditions. Interventions and targeted therapies can be tailored to minimize the effect of nanoparticles on the morbidities of chronic diseases. The majority of the protein–nanoparticle interactions augment the connection between chronic inflammation and serious medical conditions.[114] The crucial linkage between protein–nanoparticle interactions and long-term inflammation starts with the rapid formation of a protein corona on nanoparticle surfaces upon interaction with biological fluids. This fluid dynamic layer of adsorbed proteins radically transforms the nanoparticle's properties and its biological identity, determining vital biological processes such as biodistribution, cellular uptake, and interaction with immune cells.[115] In particular, protein–nanoparticle complexes can be sensed by pattern recognition receptors (PRRs) on immune cells such as Toll-like receptors (TLRs), which activate inflammatory pathways. The protein corona can also enhance phagocytic recognition and cellular uptake of nanoparticles by cells such as macrophages. Upon cellular internalization, the complexes can stimulate intracellular signalling pathways and hence enhance production and secretion of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6.[116] Likewise, protein–nanoparticle interactions lead to oxidative stress by producing reactive oxygen species, which further exacerbate the inflammatory process and are responsible for DNA damage and mutagenic activity. The long-term persistence of these protein–nanoparticle complexes within the body, possibly as a result of extended circulation or ineffective removal, can maintain this inflammatory process. This sustained inflammation, referred to as prolonged immune response, can cause tissue damage and the improper production of key cytokines that are involved in immune system homeostasis and can contribute to chronic inflammatory diseases.[117] In conclusion, this prolonged induction of inflammatory processes by protein–nanoparticle complexes is strongly associated with tissue damage, immunological disturbance, and the onset or worsening of chronic inflammatory diseases such as cancer, neurological conditions, and cardiovascular disease. It is necessary to comprehend this complicated interaction in order to understand how exposure to nanomaterials can cause or aggravate chronic conditions. (Fig. 5). For example, in relation to cardiovascular disease, inflammation due to the nanoparticles could accelerate atherosclerosis by the recruitment of immune cells, which participate in the formation of inflammatory plaques in blood vessels. Again, inflammation that is triggered by the nanoparticles could contribute to the loss of neurons and the development of cognitive deficiency that is presented in neurodegenerative diseases such as Alzheimer's.[118] Moreover, prolonged inflammation that is caused by nanoparticles that induce cancer can support tumor growth, invasion, and metastasis by the activation of a pro-tumor environment characterized by angiogenesis, immune suppression, and tissue remodelling.[119] Therefore, the detailed analysis of the biochemical pathways and immunomodulatory mechanisms mediating protein–nanoparticle interaction is crucial to understanding the complex relationship that exists between the development of many chronic diseases and chronic inflammation.

This information could explain the pathophysiology of chronic health conditions and also contribute to the development of targeted treatment strategies aimed at reducing the inflammation-related risks associated with nanoparticles.

6. Implications for chronic diseases

6.1. Cardiovascular diseases and the role of nanoparticle–protein complexes

An estimated global report reveals that cardiovascular diseases (CVDs) constitute the main cause of morbidity and death worldwide. Among the most common diseases of CVDs are atherosclerosis, myocardial infarction, and heart failure. Traditional studies have recognized protein–nanoparticle interactions as a vital gateway to the development and progression of CVDs. Nanoparticles upon coming in contact with the biological fluids form a protein corona quickly, which changes their biophysical features and interaction with biological systems.[120] The protein–nanoparticle complexes in the setting of CVD may induce acceleration of atherosclerosis by enhancing inflammation, oxidative stress, and endothelial dysfunction

within the arterial wall. The putative effects of the complexes on lipid metabolism and plaque stability lead to the formation and exacerbation of atherosclerotic lesions.[121] Protein–nanoparticle interactions may lead to thrombosis through activation of platelets and coagulation cascades, increasing the risk of myocardial infarction and stroke.[122] Nanoparticle formulations, when tailor-made, have applications in targeted drug delivery, therapeutic treatments, and imaging in CVDs. The formulations allow for precise targeting of plaques, precise modulation of vascular function, and diagnostic imaging.[123] With the complex interactions these nanoparticle–protein complexes have with the cardiovascular system, an extensive preclinical and clinical study in regard to safety, effectiveness, and long-term implications is needed. In relation to cardiovascular diseases, the development of nanomedical interventions based on such complex interactions between proteins and nanoparticles must take into account biocompatibility to minimize adverse effects and assure the safety and efficacy of cardiovascular therapies.

6.2. Neurological disorders influenced by chronic inflammation

Chronic inflammation plays a central role in neurological disorders, and the recent research indicates that the central nervous system regulates inflammatory reactions mostly by the interaction of proteins with nanoparticles. In fact, chronic inflammation has been proven to play an important role in many neurological pathologies, including Parkinson's disease, multiple sclerosis, stroke, and Alzheimer's disease.[124] In those cases, persistent activation of microglia, resident immune cells in the CNS, and infiltration of peripheral immune cells cause neuronal injury, synaptic dysfunction, and neurodegeneration.[125] Complexes of proteins with nanoparticles formed by protein adsorption onto the surfaces of nanoparticles are capable of enhancing neuroinflammation in the brain by activation of microglia, enhancement of the production of pro-inflammatory cytokines, and enhancing oxidative stress.[126] Such complexes can further facilitate neuroinflammatory responses by allowing immune cells and inflammatory mediators to pass through the blood–brain barrier into the central nervous system.[127] Targeted drug delivery to the brain, imaging of neuroinflammatory processes, and regulation of the immune response are only a few promising therapeutic possibilities offered by specific nanoparticle formulations.[128] The intricate interaction of protein–nanoparticle complexes with the central nervous system warrants a judicious examination of neurotoxicity, immunomodulation, and biocompatibility in preclinical models as well as in clinical trials.[129] The elucidation of how nanoparticle–protein complexes may contribute to neuroinflammation will enable researchers to establish new targets for treatment and innovative strategies for the therapeutic management of neurological diseases, ultimately bettering patient outcomes and quality of life. Understanding of the intricately interconnected relationship of chronic inflammation and nanoparticle–protein complexes in neurological disease is therefore critical to create targeted therapeutic approaches that reduce side effects while increasing therapeutic outcome in this intensely complex and challenging area.

6.3. Respiratory diseases and implications of long-term exposure

COPD or chronic obstructive pulmonary disease, respiratory infections, and asthma are a few illustrations of respiratory diseases that pose a serious public health risk worldwide. Particulate matter and environmental pollutants are major contributors to the development and aggravation of respiratory diseases, including asthma.[130] Previously published data show that pulmonary pathophysiology is greatly affected by nanoparticle–protein complexes that are produced when proteins bind to the surfaces of nanoparticles.[131] When particulate matter from industrial or traffic emissions, or other air pollutants that contain nanoparticles, is inhaled, the respiratory tract is coated by nanoparticle–protein complexes.[132] These complexes can be engaged through the respiratory mucosa, lung epithelial cells, and immune cells. These complexes may exacerbate respiratory inflammation through tissue injury, oxidative stress, and generation of pro-inflammatory cytokines and chemokines brought about by the activation of immune cells such as dendritic cells and macrophages.[51] Furthermore, mucus hypersecretion, altered airways, and compromised lung function aggravated the pathogenesis of respiratory diseases through nanoparticle–protein complex formation.[133] Persistence of such complexes in the respiratory tract can prolong the course of diseases through the perpetuation of chronic inflammation and increased susceptibility to respiratory infections.[134] On the other hand, disease management in the respiratory tract can take advantage of customized nanoparticle formulations that allow for precise control over inflammatory reactions and tissue regeneration for targeted administration of drugs, imaging, and therapeutic interventions.[135] The immunomodulatory effects and pulmonary toxicity and biocompatibility of nanoparticle–protein complexes have to be carefully assessed to ensure safe and efficient application of such complexes in respiratory medicine. Dynamics of the interaction between nanoparticles and proteins in the respiratory system are critical to determine the long-term health effects of environmental exposure and produce low-respiratory toxicity nanoparticles for various industrial and medicinal applications.

6.4. Metabolic disorders and their effects on insulin resistance

Obesity, type 2 diabetes mellitus, and metabolic syndrome have become an epidemic or threat worldwide. The pathology of these disorders is known to be characterized by poor energy metabolism, insulin resistance, and low-grade, chronic inflammation. Insulin resistance is a common feature of these disorders, resulting in elevated blood glucose levels and reducing the body's ability to absorb glucose through peripheral tissues.[136] Thus, recent studies indicate that nanoparticle–protein complexes greatly contribute to insulin resistance and metabolic dysfunction. Complexes can interact with hepatocytes, adipocytes, and skeletal muscle cells, modulating insulin signalling pathways and triggering inflammatory responses. These are the complexes formed by protein adsorption on the surfaces of the nanoparticles

themselves.[137] Glucose intolerance and insulin resistance can be caused by oxidative stress, pro-inflammatory signaling pathways, and inhibition of insulin receptor signalling by nanoparticle–protein complexes.[124] Insulin resistance and metabolic disturbances have been further associated with exposure to environmental nanoparticles for an extended period of time, like particulate matter from air pollution.[138] On the other hand, some nanoparticle formulations provide possibilities for targeted drug delivery, therapeutic interventions, and imaging in metabolic diseases, enabling accurate regulation of insulin sensitivity and metabolic pathways. Nevertheless the potential harm that nanoparticle–protein complexes may cause to metabolic health underscores how important it is to thoroughly investigate their immunomodulatory, metabolic toxicity, and biocompatibility. For the safe design and use of nanoparticles in drug delivery systems or other biomedical applications that aim to limit deleterious affects on metabolic function, an understanding of the underlying processes of nanoparticle–protein interactions in the context of metabolic health is essential.

6.5. Autoimmune conditions and the potential triggering by nanoparticles

The immune system attacking its own tissues is the result of dysregulated immunological reactions, which include autoimmune diseases including lupus, rheumatoid arthritis, and multiple sclerosis. Recent research suggests that nanoparticles and nanoparticle–protein complexes may cause or possibly aggravate autoimmune diseases. After entering the body *via* a variety of routes, such as ingestion, injection, or inhalation, nanoparticles engage with immune cells and tissues.[139] When exposed, protein complexes including nanoparticles can influence immune responses by generating a protein corona. These combinations have the power to activate autoreactive T and B cells and stimulate immunological cells including dendritic cells and macrophages, which in turn causes the release of pro-inflammatory cytokines.[140] Alternatively, immunological tolerance may be compromised and autoimmune reactions may arise as a consequence of oxidative stress and tissue injury caused by nanoparticles. Furthermore, altered compositions of nanoparticles, such as those used in the delivery of medicines and imaging, are able to change immune responses and can be used in the management of autoimmune diseases.[141] Nanoparticle–protein complex immunomodulatory effects need to be carefully examined to prevent autoimmune response amplification. The immunomodulatory potential of nanoparticle–protein complexes needs to be realized in assessing efficacy for nanomaterials across applications and developing means to reduce risks of autoimmune disease initiation or advancement.

6.6. Cancer development and progression influenced by chronic inflammation

Inflammatory responses strongly influence the establishment and spread of chronic inflammation, thus significantly facilitating tumour genesis, propagation, and metastasis. A recent study revealed that nanoparticle–protein complexes impact the chronic inflammation in the tumor microenvironment and consequently influence the cancer progression.[142] Nanoparticles accumulate in tumors mainly through two mechanisms: the enhanced permeability and retention (EPR) and active targeting, where particles interact with proteins, forming protein coronas.[143] These protein–nanoparticle complexes, after the activation of immune cells, like dendritic cells and tumor-associated macrophages, release growth factors, chemokines, and proinflammatory cytokines, which promote tumour growth and invasion. Furthermore, oxidative stress and DNA damage caused by nanoparticles could accelerate the formation of tumours and genomic instability.[144] Engineered nanoparticle formulations, on the other hand, offer a promising approach for cancer detection, imaging, and therapy, with applications including targeted drug delivery, photothermal therapy, and immunomodulation (Table 6). But the immunomodulatory effects of nanoparticle–protein complexes in the tumour microenvironment must be carefully monitored since they can either improve anti-tumor immune responses or increase immune evasion and tumour growth. Understanding the complex interplay between nanoparticle–protein complexes and chronic inflammation in cancer development and progression is critical for developing effective cancer therapies and personalised treatment strategies that target tumor-associated inflammation while minimising the side effects on normal tissues (Fig. 6).

7. *In vivo* and clinical studies

In longitudinal studies for the evaluation of nanoparticle exposure, systematic observation of individuals is made for an extended period of time in order to fully understand the effects of continuous exposure to nanoparticles on health. It is possible to monitor the biodistribution, clearance, and possible toxicity of nanoparticles over time by using imaging methods, biomonitoring, and clinical evaluations.[153] These studies are essential for providing information on cumulative health consequences, highlighting possible dangers associated with chronic exposure and clarifying the dynamic interactions between nanoparticles and biological systems (Table 7). The goal of epidemiological research connecting nanoparticle exposure to chronic diseases is to identify correlations between the onset or worsening of chronic illnesses in human populations. These large-scale population studies evaluate associations between the occurrence of illnesses including respiratory disorders, cardiovascular diseases, or inflammatory conditions with exposure to environmental or occupational nanoparticles. Understanding the practical consequences, identifying individuals at risk, and guiding public health policy about nanoparticle exposure all depend heavily on epidemiological information. Translating the research findings from observational and laboratory investigations into useful uses for clinical settings is known as clinical implications and translational aspects. It is feasible to establish targeted treatments, diagnostic techniques, and treatment plans, by studying the relationship between clinical outcomes and nanoparticle exposure as seen in epidemiological and longitudinal research. Ultimately, this translational aspect helps to enhance patient outcomes

Table 6 Clinically approved nanomedicines and advanced clinical trials

Drug name (formulation)	Treatment(s)	Nanotechnology aspects	Source(s)
Doxil (liposomal doxorubicin)	Ovarian cancer, Kaposi's sarcoma, and multiple myeloma	PEGylated liposomes encapsulate doxorubicin, reducing cardiotoxicity and enhancing tumor targeting *via* the enhanced permeability and retention (EPR) effect	145
Abraxane (nab-paclitaxel)	Breast cancer, non-small cell lung cancer, and pancreatic cancer	Albumin-bound paclitaxel nanoparticles improve solubility and facilitate transport across tumor vasculature *via* albumin receptors (*e.g.*, SPARC)	146
Onivyde (liposomal irinotecan)	Metastatic pancreatic cancer	Liposomal formulation enhances the delivery of irinotecan to tumor tissues, prolonging circulation time and improving therapeutic outcomes	147
Ferumoxytol (feraheme)	Iron replacement therapy for anemia and contrast agent for MRI	Superparamagnetic iron oxide nanoparticles provide both therapeutic and diagnostic benefits, showcasing the potential of theranostics	148
mRNA Vaccines (*e.g.*, Pfizer-BioNTech, Moderna)	Vaccination against infectious diseases (*e.g.*, COVID-19)	Lipid nanoparticles (LNPs) shield mRNA from enzymatic degradation and enhance its cellular uptake, enabling robust immune responses	149
BIND-014 (docetaxel-loaded nanoparticles)	Targeted delivery of docetaxel to prostate cancer cells	Targeted delivery of docetaxel to prostate cancer cells using PSMA-targeting ligands	150
CRLX101 (cyclodextrin-based nanoparticles)	Treatment of solid tumors	Cyclodextrin-based nanoparticles deliver camptothecin to treat solid tumors, currently in phase 2 trials	151
NU-0129 (spherical nucleic acids)	Delivery of siRNA to glioblastoma	Gold nanoparticle-based platform delivers siRNA to glioblastoma, advancing through early-phase trials	152

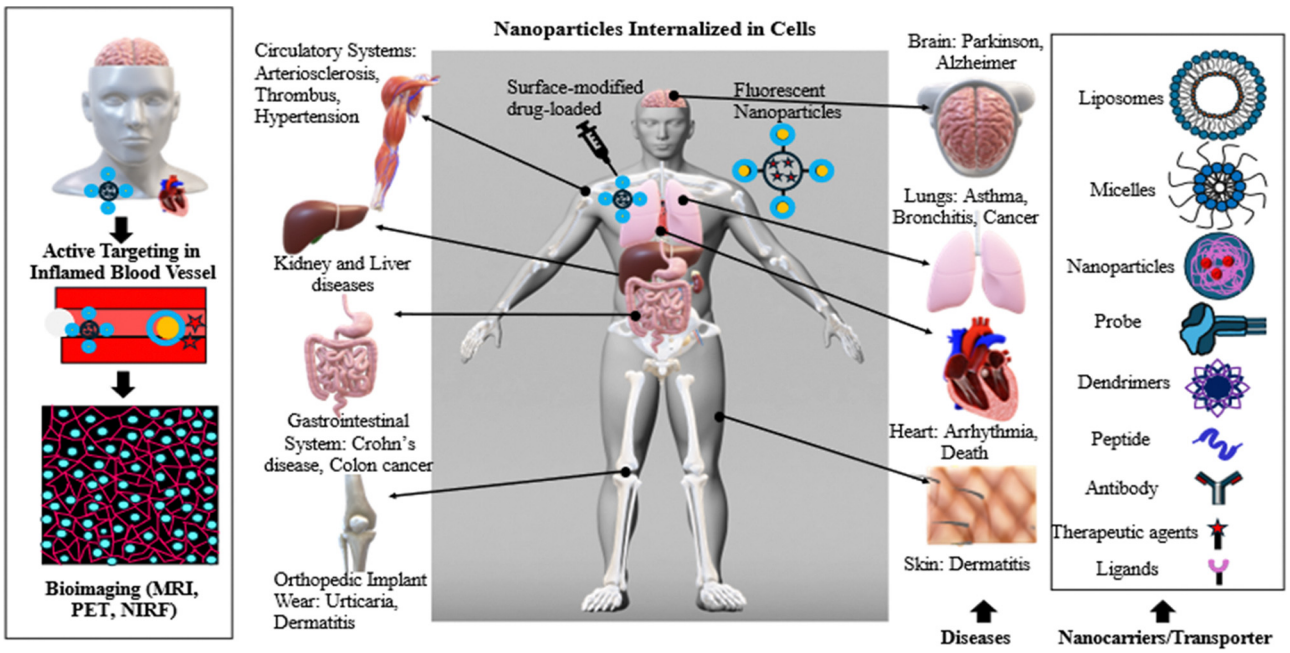

Fig. 6 Nano–protein interactions and their implication in chronic diseases.

and produce guidelines for managing possible health concerns associated with nanoparticle exposure by bridging the gap between scientific research and its use in healthcare.

8. Genotoxicity and immunotoxicity

8.1. DNA damage and mutagenic effects

A challenging and concerning feature of nanotoxicology and medicinal applications is the mutagenesis effects and damage to DNA caused by protein–nanoparticle complexes.[159] Nanoparticles quickly acquire a protein corona through coming into contact with biological systems, which changes their physicochemical characteristics and biological behaviour. By producing reactive oxygen species (ROS), these complexes can cause oxidative stress (Fig. 7). ROS can then cause base alterations, single-strand breaks, and double-strand breaks, among other types of DNA damage.[160] Furthermore, direct interactions between DNA and protein–nanoparticle complexes can result in physical harm and disruption of DNA repair pathways.[4] Prolonged interaction with these complexes enhances DNA damage and elevates the chances of

Table 7 A list of comprehensive longitudinal studies provides a framework for tracking the biodistribution, clearance, and potential toxicity of nanoparticles with deeper understanding of their impact on health

Aspect	Description	Findings	Ref.
Imaging techniques	Utilization of various imaging modalities such as FTIR, XRD, and fluorescence imaging to visualize the biodistribution of nanoparticles *in vivo* allows for real-time tracking of nanoparticle accumulation in organs, tissues, and cells	Incorporating MgO into mesoporous carbon nitride (MCN) improved CO_2 uptake efficiency and enhanced textural properties. Gas chromatograph analysis showed the efficiency of the sorbents. Formation of $MgCO_3$ indicated bulk chemical phase conversion	154
Biomonitoring	Monitoring of biological samples (blood, urine, and tissues) for nanoparticle presence, metabolites, and biomarkers of toxicity or clearance provides insights into nanoparticle absorption, distribution, metabolism, and excretion pathways over time	Analysed various entry routes of nanomaterials in the human body and explored the passage of nanomaterials into air, water, and soil ecosystems	155
Clinical assessments	Evaluation of physiological parameters (*e.g.*, organ function and blood chemistry) and clinical outcomes (*e.g.*, symptoms and adverse effects) following nanoparticle exposure enables the assessment of systemic effects, potential toxicity, and long-term health implications associated with nanoparticle exposure	Gold-based photosensitive nanomaterials can specifically excite pyramidal neurons in the hippocampus. Transcranial photo-biomodulation improves cognitive function in healthy individuals. NIR laser therapy shows promise for targeted drug delivery; graphene-based materials have potential applications in treating neurocognitive diseases	156
Longitudinal sampling and follow-up	Regular collection of biological samples and clinical data at predefined intervals over an extended duration to monitor changes in nanoparticle distribution, clearance, and toxicity Allows for the assessment of temporal trends, individual variability, and cumulative effects of nanoparticle exposure	Serial blood sampling approach helps control over inter-animal variability. Offers cost savings for non-rodent species and specialized disease. PET and SPECT imaging techniques offer accurate quantification of nanomaterial distribution in blood	157
Integration of multimodal data	Integration of imaging, biomonitoring, and clinical assessment data to correlate nanoparticle biodistribution, clearance kinetics, and toxicity profiles over time Provides a comprehensive understanding of the dynamic interactions between nanoparticles and biological systems, aiding in the interpretation of long-term health effects	Useful insights into the mechanisms of NP pharmacokinetics, revealing the key mechanisms for the AuNP absorption routes; clarified the key mechanisms for the inhaled AuNP biodistribution to secondary organs	158

mutagenesis and genomic instability.[161] Thus, from this perspective, comprehension of the mechanism underlying the mutagenesis and DNA damage mediated by protein–nanoparticle complexes is essential to establishing the safety of nanomaterials and developing mitigating measures for their genotoxicity in biological applications.

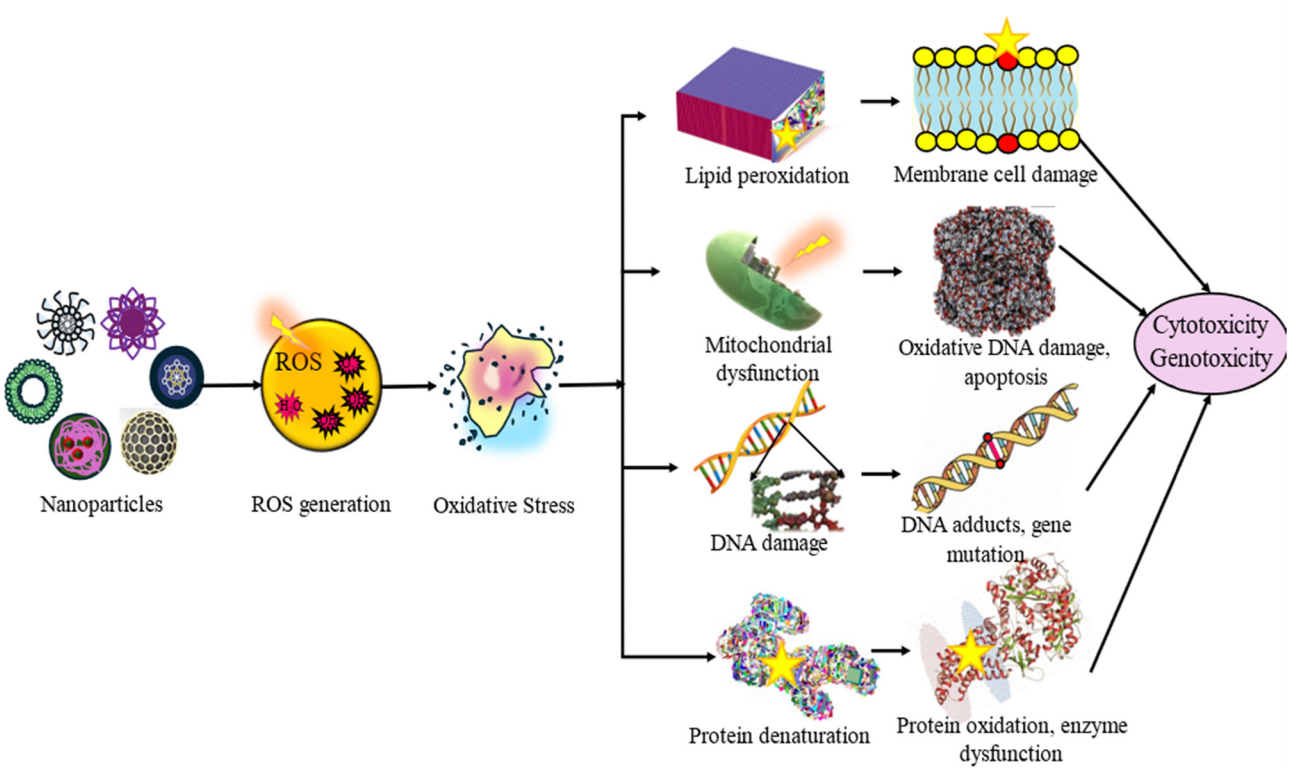

Fig. 7 Toxic effects induced by protein–nanoparticle complexes.

8.2. Immunomodulatory responses and their implications for immune function

These protein–nanoparticle complexes have immense effects on immunological function and general health through such immunomodulatory responses The protein corona quickly formed by the interaction of nanoparticles with biological systems has the ability to affect immune recognition and the consequent reaction.[162] Such complexes can activate a number of immunomodulatory pathways, such as the regulation of cytokine production, modification of immune cell functions, and activation of inflammatory cascades. Immunomodulatory reactions may involve activation, proliferation, and differentiation in a pro- or anti-inflammatory way, depending on the composition, size, and surface characteristics of the nanoparticles.[163] Protein–nanoparticle complexes can influence antigen presentation and immune cell trafficking, which may influence adaptive immune responses. Long-term exposure to these complexes may result in immunological dysregulation, exacerbating or initiating autoimmune diseases, allergic reactions, and inflammatory disorders. On the other hand, protein–nanoparticle complexes that have been created show potential for immunomodulatory treatments, including immunotherapy, vaccine development, and targeted drug delivery.[164] One of the most notable applications is their utilization in the development of cancer vaccines, *e.g.*, mRNA lipid nanoparticle (LNP) vaccines, which gained popularity during the COVID-19 pandemic and are now being developed for oncology. These LNPs are delivery vehicle-like systems that protect mRNA from enzymatic degradation and enhance targeted delivery to antigen-presenting cells and immune responses by inducing robust T-cell activation and tumor-selective immunity. Besides mRNA vaccines, nanoparticles are also being engineered for broad immune modulation, enabling dampening of overactive immune responses in autoimmune diseases or induction of anti-tumor immunity in cancer treatment. By altering surface properties, incorporating specific ligands, or encapsulating immunostimulatory molecules, nanoparticles can selectively modulate immune cells, regulate cytokine production, and modulate signaling pathways, with immense potential for personalized immunotherapy.[165] The absence of an in-depth discussion of such groundbreaking applications restricts the scope of the review, given their far-reaching potential for advancing precision medicine, addressing unmet clinical needs, and transforming therapeutic paradigms for both cancer and immune disorders. Nanoparticles have transformed CRISPR-Cas9 and gene therapy by enhancing the delivery, stability, and effectiveness of gene-editing elements. Conventional viral vectors entail risks such as immune reaction and insertional mutagenesis, while nanoparticles provide a safer, non-viral option for accurate genome editing. Lipid, polymeric, and inorganic nanoparticles improve cellular uptake and preserve the CRISPR-Cas9 cargo from degradation to facilitate effective gene correction in conditions such as genetic disorders, cancer, and viral infections.[166] Modern improvements center on controlled nanoparticle platforms that increase selectivity, lower off-target responses, and permit *in vivo* editing of genes to set the stage for personalized and regenerative therapeutics (Table 8) Understanding the complicated interaction between protein–nanoparticle complexes and the immune system is crucial for optimising their medicinal possibilities while reducing unfavourable immune responses, thus propelling the domains of nanomedicine and personalised medicine.

9. Mitigation strategies

9.1. Surface modifications for enhanced biocompatibility

Surface modifications are a common target of mitigation efforts to improve the biocompatibility of nanoparticles by reducing unfavourable biological interactions and increasing their compatibility with biological systems. One method is to coat the nanoparticles with biocompatible polymers, such polyethylene glycol (PEG), which forms a hydrophilic layer that decreases immunological recognition and increases circulation time by lowering protein adsorption and opsonization. To further improve biocompatibility, surface functionalization with zwitterionic compounds or stealth coatings can provide a neutral charge and inhibit protein adsorption.[167] Targeting ligands, such peptides or antibodies, can be added to the surface of

Table 8 Nanoparticles in CRISPR-Cas9 and genetic treatments

Aspect	Details	References/examples
Targeted delivery	Nanoparticles can be engineered to deliver CRISPR-Cas9 components (Cas9 protein and sgRNA) to specific tissues or cells, reducing off-target effects	Lipid nanoparticles (LNPs) and gold nanoparticles for liver and cancer targeting
Enhanced stability	Nanoparticles protect CRISPR-Cas9 components from degradation by nucleases and proteases in the bloodstream	Polymeric nanoparticles (*e.g.*, PLGA) and lipid-based systems
Improved cellular uptake	Surface modifications (*e.g.*, PEGylation and cell-penetrating peptides) enhance cellular internalization of CRISPR-Cas9	Peptide-modified nanoparticles for efficient delivery to neurons and stem cells
Reduced immunogenicity	Nanoparticles can shield CRISPR-Cas9 components from the immune system, minimizing adverse reactions	PEG-coated nanoparticles to evade immune detection
Controlled release	Nanoparticles enable sustained or stimuli-responsive release of CRISPR-Cas9, improving precision in gene editing	pH-sensitive or redox-responsive nanoparticles for tumor-specific delivery
Versatility	Nanoparticles can deliver various forms of CRISPR-Cas9, including plasmid DNA, mRNA, and ribonucleoprotein (RNP) complexes	Gold nanoparticles for RNP delivery; LNPs for mRNA delivery
Applications in gene therapy	Nanoparticles enable CRISPR-Cas9 delivery for treating genetic disorders, cancers, and infectious diseases	LNPs for sickle cell anemia; polymeric nanoparticles for cystic fibrosis gene editing
Challenges	Potential toxicity, scalability, and long-term effects of nanoparticle-based delivery systems need further investigation	Studies on biocompatibility and biodegradability of nanoparticles

nanoparticles to enable targeted interactions with target cells or tissues, reducing the likelihood of off-target effects and enhancing therapeutic efficacy. Controlled drug release in response to physiological signals is also made possible by developing nanoparticles with stimuli-responsive coatings, which improves therapeutic effects while lowering systemic toxicity.[168] Cell-penetrating peptides or endosomal escape moieties may also be attached to the surface to facilitate cellular absorption and intracellular distribution of therapies.[169] In addition, the creation of multifunctional nanoparticles which integrate therapeutic loads, targeting moieties, and imaging moieties enables precise and targeted medical treatment in diverse disease conditions. The surface modifications are comprehensive in bestowing biocompatibility on the nanoparticles, thus enabling their safe and efficient use in biomedical applications such as imaging, regenerative medicine, drug delivery, and diagnostics.

9.2. Design strategies for biocompatible nanoparticles

A key initial step towards developing biocompatible nanoparticles involves selecting materials with intrinsic biocompatibility, such as lipids or biodegradable polymers; these characteristics minimise possible negative effects and enhance their potential for a range of applications. These substances are frequently well absorbed by the human body and may be made to degrade under controlled conditions, which lowers exposure over an extended period of time.[170] Furthermore, a nanoparticle's biocompatibility is greatly influenced by its size and shape. Because they could evade the immune system, small-sized nanoparticles are typically used for drug delivery applications. However, properly designed geometries can affect cellular absorption and dispersion within the body.[171] Another key strategy for making biocompatible nanoparticles is surface modification. Biocompatible molecules such as polyethylene glycol (PEG) or other hydrophilic polymers may cover nanoparticles to avoid protein adsorption and the creation of a corona that could lead to an immune response.[172] By adding certain ligands to the surfaces of nanoparticles, one can enable targeted transport to particular cells or tissues, increasing therapeutic efficacy and reducing off-target effects.[173] An additional level of complexity to improve biocompatibility is added by using responsive or "smart" nanoparticles that could adjust to the physiological milieu and release therapeutic payloads in a regulated way. In order to obtain the ideal biocompatibility for a variety of biomedical applications, a thorough approach to nanoparticle design takes into account the interaction between material selection, size, shape, surface characteristics, and functionalization.

10. Future directions in research

10.1. Advanced *in vitro* models and organ-on-a-chip systems

Research on nanoparticle interactions is expected to make significant advances in the future through the development and use of sophisticated *in vitro* models and organ-on-a-chip systems. Better than traditional cell culture models, these cutting-edge platforms replicate the complex microenvironments of tissues and organs under well maintained laboratory conditions. They also offer substitutes that are physiologically viable and more accurate. The microfluidic system of organ-on-a-chip can replicate the structural and functional features of the liver, heart, brain, lung, and other organs. It provides a platform to study the interaction of nanoparticles within a tissue environment.[174] These systems offer the possibility of controlling variables of fluid flow, shear stress, and cell–cell interactions, which make the study of nanoparticle behavior under dynamic physiological conditions possible. Integration of such organ-on-a-chip systems with advanced imaging techniques, like high-resolution microscopy and live-cell imaging, enables the real-time monitoring and analysis of the dynamics of nanoparticles in living tissues.[175] In addition, the development of organotypic co-culture models incorporating different cell types and tissue components provides a deeper understanding of the interaction between nanoparticles in complex biological systems. These sophisticated *in vitro* models may be used not only for the evaluation of nanomaterial toxicity or its uses in drug administration but also for enhancing our mechanistic understanding of nanoparticle behavior. All this advances personalized healthcare.[176,177] Therefore, the use of such advanced *in vitro* models and organ-on-chip systems in nanoparticle studies can greatly contribute to the acceleration of scientific research, enhancing safety and efficiency, and bringing nanomedicine closer to clinical application.

10.2. Emerging technologies in nanoparticle research

Such technological development is crucial to new knowledge and new opportunities for nanoparticle research; the field is still growing. One of the most outstanding technological developments is the creation of advanced imaging methods, including cryo-electron microscopy and super-resolution microscopy, enabling nanoparticle analysis at previously unattainable high resolutions. This provides data on the processes of what is going on in cells with the nanoparticles and detailed assessment of their interactions with the biological components[178,179] as seen in Fig. 8. In addition, advances in spectroscopic techniques, including coherent anti-Stokes Raman scattering and surface-enhanced Raman scattering, permit a sensitive assessment of the molecular interactions taking place at the nanoscale. It allows for detailed investigations into the development of protein coronas within the nanoparticles and the protein–nanoparticle interactions.[180] Another extremely promising direction in the area of nanoparticle studies is the integration of machine learning and artificial intelligence. These methods help to simplify data analysis, find patterns, build prediction models, and discover hidden relationships within large datasets.[181] *In silico* techniques, powered by machine learning algorithms, can predict the toxicity of a nanoparticle, guide the design of a nanoparticle to be suited to a certain application, and optimize formulations.

11. Ethical considerations and public perception

Increasing applications of nanoparticles in many fields, particularly in technology and medicine, raise serious ethical questions.

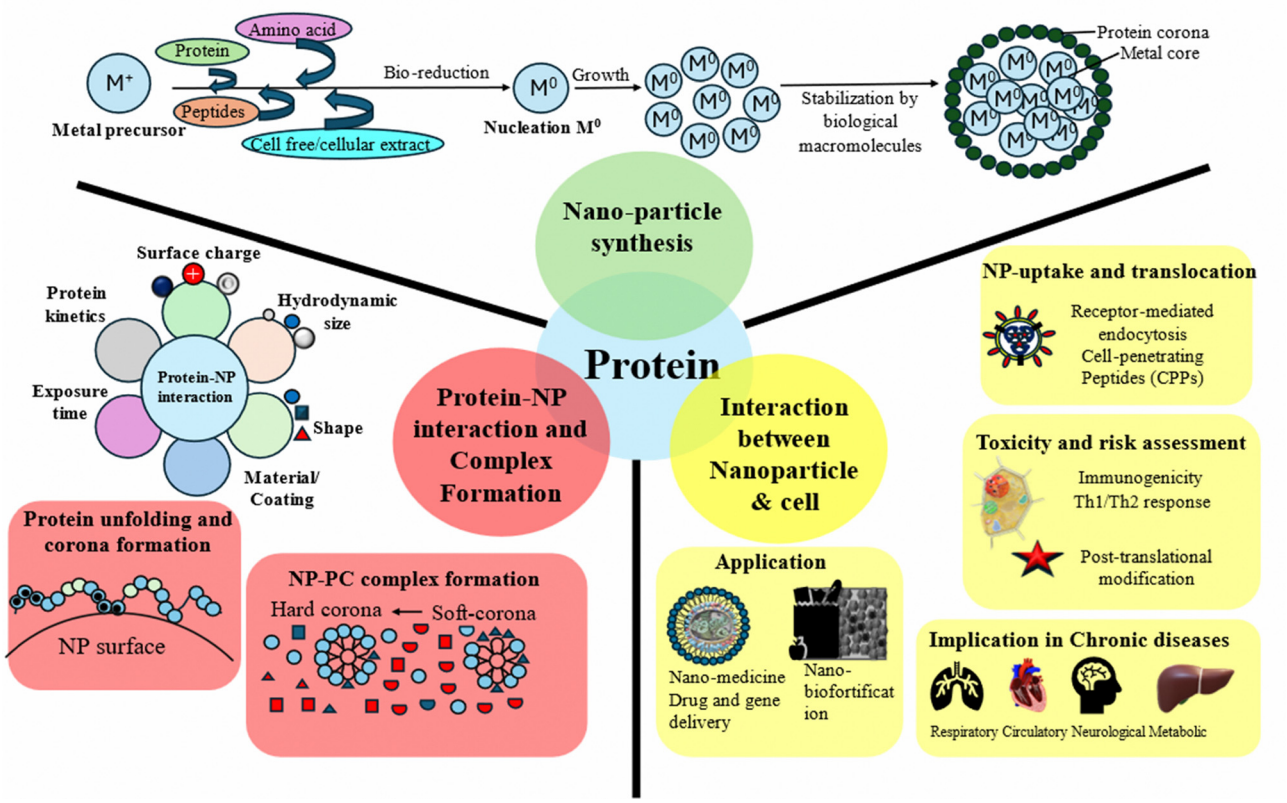

Fig. 8 Protein–nanoparticle interaction and its implications in chronic diseases.

Among the primary concerns are possible long-term effects of nanoparticle exposure on the environment and human health.[182] Protection of health and safety of humans and the environment is the first priority for both scientists and industry. The use and research of nanoparticles should strictly follow high moral standards and comprehensive risk assessment. Ethical concerns also arise regarding the risk of the distribution of resources, which can lead to unequal access to technical and medical developments, thus causing inequality in utilising the potential advantageous impacts of nanotechnology. Ethical concerns over proper disposal of nanoparticles and their effects on the environment emphasize the need for a balance between conservation and scientific development. Public opinion and understanding strongly influences the ethical uses of nanoparticles. Lack of knowledge regarding the benefits and risks of nanotechnology, or false information provided, might give rise to distrust or concern among the general public. Therefore, open communication about the disadvantages and advantages of nanoparticles is very important. Safety, environmental impact, and societal implications of nanotechnology are taken into consideration, and hence public involvement in the making of decisions is required. Ethical considerations involve ensuring that the applications of nanotechnology do not disproportionately harm underprivileged people and regulatory structures that help in protecting the health and welfare of the public. Transparent communication lines between scientists, lawmakers, business operations, and the public are needed for the establishment of ethical governance and public trust in the manufacture and use of nanoparticles.

12. Conclusion

In order to establish a thorough link between nanoparticles and the immune system with significant health consequences for humans, this review summarises research on protein–nanoparticle interactions and their long-term effects on chronic inflammation. Size, surface charge, and surface chemistry are a few of the physicochemical characteristics of nanoparticles that have been studied as being crucial in defining their immunomodulatory effects and long-term biological complications. Further debate has focused on the ways in which the protein corona affects immunological recognition, inflammatory responses, and cellular absorption, all of which eventually aid in the development and sustenance of chronic inflammation. The long-term elevation of inflammatory processes by nanoparticle–protein complexes may lead to tissue damage, immunological dysregulation, and chronic inflammatory diseases including cancer, neurological disorders, and cardiovascular disease. This review also examines the immunotoxicity and biocompatibility of nanoparticles in order to suppress any potential side effect and allow the safe and efficient use of nanomaterials in medicine. In addition, targeted therapeutic interventions for immune response modification and

diminishing the risk of inflammatory diseases due to nanoparticle exposure necessitate understanding the mechanisms of protein–nanoparticle interactions and chronic inflammation. It was shown that exposure to biological fluids could easily confer nanoparticles a protein corona, thus modifying their biological function and immunomodulatory characteristics, as noted in a recent study. In spite of tremendous progress in this field, the long-term effects of protein–nanoparticle interactions on chronic disorders are largely unknown. The fundamental mechanisms giving rise to disease and chronic inflammation following exposure to nanoparticles still harbor a plethora of unanswered questions. Future studies focusing on the exact pathways involved should be the major focus, taking into consideration individual variability, dose–response relationships, and other properties of nanoparticles. Further studies are required on the potential effects of chronic nanoparticle exposure on various organ systems and the interactions between genetic predisposition and nanoparticles. Research on the useful applications of ethical nanomaterial design concepts that consider immunomodulatory effects and biocompatibility is insufficient. Appropriate paths for the translation of research findings into clinical and regulatory frameworks must also be developed in order to assure the safe and ethical implementation of nanoparticles in a variety of fields, including consumer products and medicine. Future multidisciplinary cooperation, moral debates, and persistent public engagement will influence the study of protein–nanoparticle interactions and their implications for chronic disorders.

Data availability

This review does not include any original research results, software, or code, nor were any new data generated or analyzed. Additional information about this review is available upon request from the authors.

Conflicts of interest

There are no conflicts to declare.

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