Reviewing Nanoplastic Toxicology: It's an Interface Problem

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Abstract

Multiple international agencies have recently raised environmental and health concerns regarding plastics in nanoforms (nanoplastics), but there is insufficient knowledge of their properties to allow for an accurate risk assessment to be conducted and any risks managed. For this reason, research into the toxicity of nanoplastics has focused strongly on documenting their impacts on biological organisms. One scope of this review is to summarise the recent findings on the adverse effects on biological organisms and strategies which can be adopted to advance our understanding of nanoplastic properties and their toxicity. Specifically, a mechanistic approach has already been employed in nanotoxicology, which focuses on the cause-and-effect relationships to establish a tool that predicts the biological impacts based on nanoparticle characteristics. Identifying the chemical and biological bases behind the observed biological effects (such as *in vitro* cellular response) is a major challenge, due to the intricate nature of nanoparticle-biological molecule complexes and an unawareness of their interaction with other biological targets, particularly at interfacial level. An exemplary case includes protein corona formation and ecological molecule corona (eco-corona) for nanoplastics. Therefore, the second scope of this review is to discuss recent findings and importance of (for both non-plastic and plastic nanoparticles) coronae formation and structure. Finally, we discuss the opportunities provided by model system approaches (model protein corona and lipid bilayer) to deepen the understanding of the above-mentioned perspectives, and corroborate the findings from *in vitro* experiments.

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Highlights:

- Nanoplastics disrupt the ecological function of biofilms, causing adverse effects in aquatic organisms, and bioaccumulate.
- The strategy adopted in non-plastic nanotoxicology field is critically discussed, and considerations specific to nanoplastic field is discussed.
- There is a major knowledge gap regarding corona formation (both protein and eco-corona) around nanoparticles, especially nanoplastics, particularly at interfacial level.
- Cellular interactions with nanoplastics (and nanoplastic-corona complexes), at interfacial level are important to understand, and the use of model membranes allows corroboration with observed *in vitro* effects

Contents

1. Introduction

Plastics in nano-scale (nanoplastics) are visually less impactful than their bulk forms or microplastics, yet, their small size makes them more challenging to remediate and facilitates their entry into biological systems, past innate defence mechanisms.[1] The World Health Organisation (WHO) noted that there was a lack of evidence that microplastics in drinking water cause significant human health problems, but could not reach a conclusion about the safety of nanoplastics due to the insufficient number of studies.[2] With increasing awareness around the globe, the nanoplastic research field emerged in the area of environmental science, investigating the origin and distribution of nanoplastics.[3-5] There is now a major focus on investigating the biological impacts of nanoplastics, in line with concerns raised in major reports by government agencies.[6-8] The first part of this review reports on the updated knowledge of the potential adverse effects of nanoplastics on biological organisms. It also describes the current mechanistic approach taken in the nano-toxicological field to better understand the cause-and-effect. Second scope of the review includes the findings on corona formation, structure, and importance of considering this to explore the underlying mechanisms of nanoplastic toxicology. Finally, it critically discusses the strategy that interfacial scientists use to fill in the knowledge gap and contribute to the mechanistic approach which can also be applied to the nanoplastic field.

Global plastic production has increased dramatically since the 1950s.[9] Concerns regarding marine plastic pollution were first raised in the 1970s, in response to their mass production and careless disposal.[10] Today, the international production of plastics exceeds 320 million tonnes per year and the growth in plastic manufacture is projected to double in 20 years, in the absence of further restrictions and altering the habit of plastic usage.[11, 12] The release of plastic from landfills into the ocean was estimated to be around 10 million metric tonnes in 2010, increasing by an order of magnitude by 2015.[13] The excessive spread of plastics has led to their unexpected discovery in places with small human influence, including the Mariana Trench, Antarctica and the Arizonan deserts.[14-17]

The plastics released in the environment undergo dynamic chemical and physical changes; photo-oxidation, slow biodegradation, and physical weathering can reduce their size range to the microplastics, and eventually, the nanoplastics, boosting their accumulation in the environment[18-21]. Increasingly, researchers have realised the impact of plastic size on environmental accumulation and potential toxicity to living organisms[17, 22-25]. Although

multiple studies have shown the potential toxicity of microplastics[23, 25, 26], few studies have compared the impact of nanoplastics to microplastics (Figure 1.1). Since the characterisation of plastic particles in the environment is only emerging, a rigorous definition of the term "nanoplastic" is yet to be established.[27] By extrapolating the definition of non-plastic nanoparticles[28], some authors have defined the size of nanoplastics to be in the range of 1 nm to 100 nm[29, 30]. Many authors set the upper size to 1000 nm[31-36], following the meaning of the prefix “nano”. The latter system of nomenclature (1-1000 nm) is followed in this review.

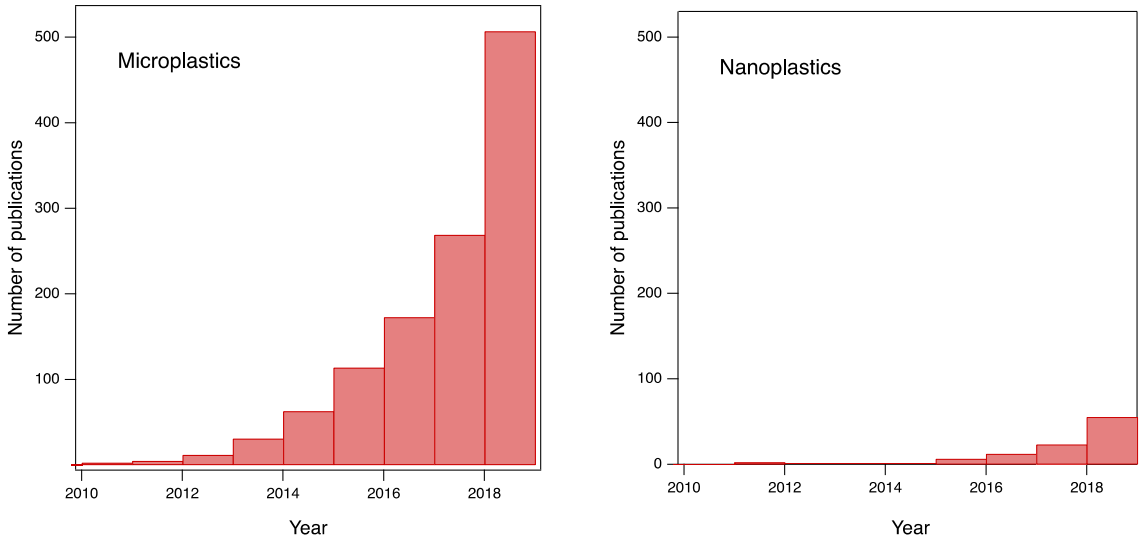

Figure 1.1. Number of journal articles published per year containing the keyword “microplastics” (left) or “nanoplastics” (right) from 2010-2019. This data was acquired from Web of Science (www.webofknowledge.com, data accessed on 15 Sep 2020).

Nanoplastic pollution is thought to occur from the careless release of waste products (primary micro/nano plastics), including pigments, cleansing scrubs, cosmetic products, and textile fibres into aquatic environments – nanoplastics emitted as a by-product of 3D printing are a new growing concern, considering the popularity of 3D printers.[5, 22] Secondary micro/nanoplastics, which result from the degradation of bulk plastics, are also thought to be the source of micro/nano plastics in the environment.[10-13, 26-28] For instance, the fragmentation of polystyrene down to the nanoscale can occur within four weeks inside a weathering chamber[21]. A recent study[49] also highlighted the fact that micro- and nanoplastics occur by mechanical milling of agricultural plastics. Normal waste water treatment systems are unable to separate nanoplastic waste from water, allowing it to pass through to rivers and oceans.[37]

In response to this growing environmental threat, a number of studies have been conducted in recent years. The German Federal Institute (GFI), in 2016, requested the European Food Safety

Authority (EFSA) to critically assess the presence of microplastics and nanoplastics in seafood.[38] Despite the large number of reports on microplastics, no information existed about nanoplastics found in commercial goods.[39-44] More recently, Wang *et al*.[45] reviewed the micro- and nanoplastics found in food chains and their implications for human health. However, few of these studies directly observed nanoplastics in the environment and in the consumer goods. The scarcity of reporting on nanoplastics arises, in a large part, from the technical and analytical challenges, *e.g.,* the small contrast between nanoplastics and food matrices when using imaging techniques. The development of nanoplastic detection techniques in seafood is a current challenge.[38]

2. Interactions with biological organisms

In comparison with their bulk analogues (bulk plastics), nanoplastics (and nanoparticles, more generally) are uniquely elusive to biological defence systems, including barriers such as tissues, mucous, and cell membranes.[46, 47] Numerous factors (e.g., particle size, elemental composition and surface groups) affect their likelihood of crossing biological barriers, including the nature of interactions.[47-50] Biological entities of different levels of complexity can be affected, with examples including biofilms[51], marine organisms[52-71], mammals[72], and humans[73-76].

2.1. Nanoplastics in bacteria and biofilms

With the wide spread of the nanoplastics in the aquatic environments and abundance, bacteria have been target organisms to study. Bacteria play important roles in essential nutrient cycles and carbon fixation.[77, 78] The study of the bacterial interactions with nanoplastics (and microplastics) is also motivated by their frequent use as an indicator in assessing ecotoxicology.[79]

Association of nanoplastics and microplastics have been correlated with harming the functionality of bacteria[80-82] and eco-toxicity[81, 83], although causes remain unclear. Miao *et al.*[81] reported the ecotoxicity is dependent on the polystyrene plastic particle size. With the size range tested in their study (100 nm – 9000 nm), only negligible effects (such as generation of reactive oxygen species (ROS)) were observed for large particles (500 nm and larger). In other work[83], the surface group of nanoplastics (100 nm) showed stronger toxicity to the biofilm compared to the ones with negatively charged surface. Notably, the biofilm formation was shown to be surface group dependent, and the extent and the trend of which group showed a stronger potent was specific to particular bacterial species. It has been known that the positive

charge is an important characteristic to target the negatively charged bacterial membrane, as demonstrated in development of antibacterial peptides.[84] However, drawing a parallel comparison may be too simplistic with limited understanding of the mechanism. Careful assessments at different biological complexity levels (from simple lipid bilayer to *in vivo* experiments) are essential in identifying the underlying causes (namely, which of nanoplastic properties are important in causing the bacterial toxicity).

2.2. Nanoplastics in marine organisms

Adverse effects on marine organisms have been documented since the early stages of nanoplastic research.[5, 85, 86] A frequently used model organism, *D. magna*, demonstrated malformation of body parts[87] and impaired reproduction[26] as a result of interaction with polystyrene nanoplastics. Liu *et al*. [88] also showed that the adverse effects caused by polystyrene nanoplastics on *D. magna* persisted over generations.

Aquatic invertebrates, such as bivalves[68, 89] and crustaceans,[90] are other frequently used model organisms. Reports indicated that exposure to functionalised polystyrene nanoplastics led to a decline in fertilisation and embryogenesis of Pacific oysters[89] and deformed larval phenotypes of blue mussel.[68] The toxicity on their gametes and embryos was demonstrated (with EC_{50} = 4.9 μg mL^{-1} and 0.15 μg mL^{-1}, respectively), although microplastics showed limited effects. Similarly, for crustaceans, developmental alteration has been reported.[59]

Fish have been common targets for studying nanoplastic toxicity, as highlighted in recent reviews.[66, 91-94] Of the biological impacts, notably, bioaccumulation has been demonstrated – polystyrene (PS) nanoplastics can propagate through a model food chain.[87, 95] When the PS nanoplastics reached the higher trophic level tested (fish), behavioural disorder was observed attributing to neurotoxicity.[87, 96] Intriguingly, almost all PS nanoplastics affected the brain function of the fish in different ways, including the cationic PS nanoplastics, which researchers previously believed had much shorter lifetimes inside biological media.[97] As with other biological organisms, underlying mechanisms of nanoplastic toxicity is not fully understood. However, there has been studies[98-100] demonstrating oxidative stress has been linked to underlying toxicity mechanisms. In addition to the toxicity, studies[101, 102] have also shown nanoplastics alter the nutritional metabolism by fish. Above all, these studies highlighted that nanoplastic concentrations can be considerable when reaching higher trophic levels despite low environmental concentration; that nanoplastics possess the ability to pass through the brain/blood barrier; and that effects may pass on to offspring.

2.3. Nanoplastics and human health

Much of the understanding around the effect of nanoplastics on human health originates from *in vitro* experiments and extrapolations from non-plastic nanotoxicology research.[73-76] Considering their ubiquitous occurrence, three plausible routes of exposure are via: (1) dermal absorption; (2) oral inhalation; and (3) ingestion. Through the use of, for example, cosmetic items applied to the skin, nanoplastics may penetrate through dermal barriers.[103] Due to the lack of experimental evidence on the atmospheric distribution of nanoplastics, studies on exposure via oral inhalation remains within occupational settings, where bulk plastics undergo mechanical and milling stress[104, 105]. Besides these, oral ingestion (likely through drinking water and food matrices) is considered the major exposure route for humans.[106] While this is plausible, there is yet to be a study experimentally confirming nanoplastic uptake from dietary contamination – although this has already been established for microplastics[104].

Following ingestion or inhalation, nanoplastics encounter mucosal barriers. Mucosal barriers play the main role in rejecting foreign objects, while maintaining efficient nutritional uptake. Nanoparticles (although not specifically nanoplastics) have been shown to be absorbed through this barrier via pinocytosis and vesicular phagocytic processes.[49] Thus far, it has been found that particles smaller than 1.0 μm have a greater tendency to be found within lymphatic tissues and their likelihood of entering the bloodstream (and ultimately, organs) is significantly higher compared to their larger analogues.[46] In particular, particles smaller than 100 nm circumvent biological barriers easily, as they are misidentify as a physiological molecule by the barriers, and make use of inherent entry mechanisms to cross them.[107]

Choi *et al*. demonstrated the translocation of various nanoparticles (CdSe, silica, and PS) from the lung to other parts of body, for a range of sizes and functional groups.[97] The study found that non-cationic nanoparticles less than 34 nm translocate from the lungs to the mediastinal lymph, and nanoparticles smaller than 6.0 nm disperse even more rapidly, reaching other organs by entering the bloodstream. For gold nanoparticles (functionalised both negatively and positively), the number of particles, sized 20 nm or below, in the bloodstream and organs increased significantly when compared with particle sizes above 80 nm.[108] Factors contributing to adverse effects.

Following the translocation and localisation of nanoplastics in specific parts of an organism, numerous biochemical events take place, which may contribute towards adverse effects either singly or in combination. Thus far, interaction with nanoplastics have resulted in the following:

alterations in gene expression[109, 110] and transcription factors[111]; oxidative stress[100, 112]; membrane damage[64]; DNA fragmentation[64]; protein modification[113]; and cytotoxicity[114].

The high surface area of nanoparticles cause excess generation of reactive oxygen species (ROS)[115]; in *in vivo* organisms (zebrafish)[100] and *in vitro* human epithelial cells[112]. Typical ROS include hydrogen peroxide, peroxynitrite, lipid hydroperoxide, and superoxide, which can damage cellular membranes, proteins, and DNA.[116]

Reproductive impairment was a major consequence of nanoplastic exposure in aquatic organisms[54, 117]. Recent studies have shown that polystyrene nanoplastics (100 nm and smaller) are able to interact with chromosomes, causing aberrations[112, 114]. Transcriptional responses have also been instigated following the interactions with nanoplastics.[110, 111] In spite of these documented biological responses, the underlying causes remain uncertain.

Table 2.1. This table summarises the biological impacts of nanoplastics are summarised and classified by different biological organisms listed in this article.

Target biological organisms and molecules	Biological effects	References
Bacteria	Enzymatic activity	80
	Toxicity	81
	Riboflavin secretion	83
	metabolism	84
Bivalves and crustaceans	Phenotype deformation	68
	Fertilization and embryogenesis	89
	Development defect	59
Fish	Bioaccumulation	87, 96
	Neurotoxicity	87, 96, 97
	Oxidative stress	98-100
	Altered metabolism	101-102

3. Predictive approach and uncovering the molecular and physical mechanism

The nanoplastic research has thus far focused on assessing the *in vivo* and *in vitro* toxicity as highlighted in this review. As with any other potential toxins, the ultimate goal is to anticipate the scale and types of hazards with the physicochemical properties of nanoplastics through structure and activity relationships (referred "predictive model" in Figure 3.1). Accurate prediction of hazards enables to identify higher risk nanoplastics and their effects, which allow informed decision-making to mitigate harm. Here, we outline the scientific challenges that should be overcome and need to be carefully considered in future research. Specifically, we

make a comparison with the progress in this field of research concerning the safety of engineered nanomaterials (hereafter, referred as the nanotoxicology).

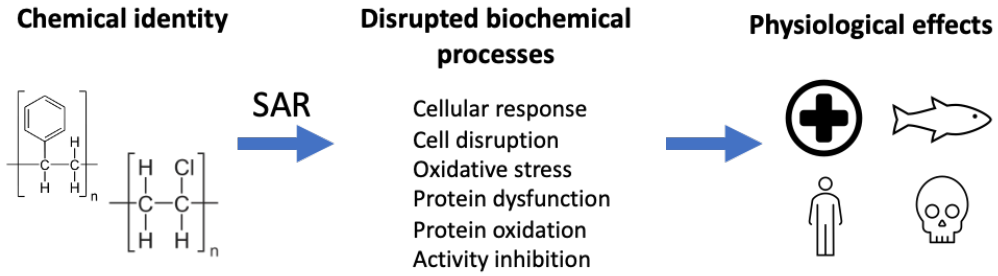

Figure 3.1. A predictive model for classical toxicology. Based on the chemical identity, structure activity relationship (SAR) predicts the affected biochemical processes, which would then anticipate the physiological effects.

In the late 2000s to early 2010s, the nanotoxicology field primarily focused on identifying the toxicological profiles using standard assays.[118] Qiu *et al.*[119] described that the next stage of the research was to understand the underlying chemical mechanisms and to establish causal relationships between the physicochemical properties of nanoplastics and the affected biochemical processes. Currently, nanoplastic toxicology is only beginning to proceed to this stage.[73] As highlighted by Qiu *et al.*[119], determining the individual contribution of each of nanoplastics physicochemical property (e.g., particle material, shapes, size, surface groups) is important, and these parameters need to be explored systematically in measuring their biological impact (e.g., cytotoxicity, ROS generation, and cellular uptake).

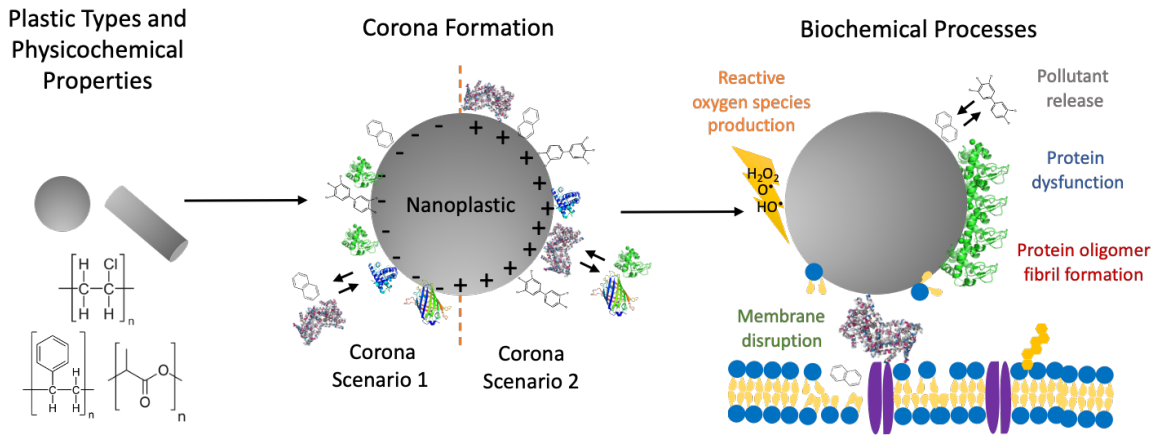

Figure 3.2. A series of events nanoplastics experience in biological organism; plastic types and physicochemical properties of nanoplastics influence the further corona formation, and further biochemical processes are determined by the nature of the nanoplastic/corona complexes.

Further, the nanoplastic surface enables the formation of complexes with macromolecules present in biological fluids, creating additional contributing factors and complicate the

establishment of the causal relationships.[120] The chemical identity and intrinsic properties of the particle affect the formation of these complexes (discussed more in detail in 4 and 4.1), and it is these complexes that determine biochemical processes.[120] A schematic of interconnected factors and a series of events, nanoplastics experience is shown in Figure 3.2. There is currently little knowledge about how the individual components of protein corona (e.g. component, shapes, protein structures, nanoplastic/corona complex,) contribute to biological interactions, and the importance of each property in this.

3.1. An important role of interface and knowledge gap

To explore the relationship between the complex of nanoplastic-biological molecules and their biological outcome (or "disrupted biochemical processes" in Figure 4.1), the formation of this complex structure has been investigated. The prediction of further interactions with other biological (macro)molecules and assemblies can however be only established with interfacial understanding.[120, 121]

Fundamentally, the interaction of nanoparticles with biological entities, e.g., a cell membrane, can be predicted by considering colloidal theories of multiple forces.[121] If the nanoparticles stay pristine on a surface, the attractive or repulsive interaction can be described by the well-known Derjaguin–Landau–Verwey–Overbeek (DLVO) theory.[122] The physical implication is that the surface character of the nanoparticles themselves dominates the colloidal behaviour (e.g., shape, size, surface charge, surface pattern). Pogodin *et al.*[123] expressed the significance of such properties by demonstrating the enhanced penetration of nanoparticles with a specific surface pattern (which may appear to be a marginal factor) through cellular membranes. As mentioned earlier, studies concerning the interfacial aspect, have infrequently considered the surface alteration due to the biological complex formation, both theoretically and experimentally.

4. Corona formation – protein and eco-molecules

The nanoparticles in biological fluid participate in the complex formation with biological molecules. The case is best exemplified by a protein corona.[120] Nanoplastics are not exempted from this scenario, and experimentally demonstrated by us in the previous work.[124, 125] Because the nanoparticle's surface properties can be altered drastically by such a surface layer, the particle's "biological identity" should consider the full complexity of the surface structure.

Both *in vitro*[126, 127] and model systems[128] have demonstrated that the formation of such nanoparticle/biological molecule complexes affect the biological interactions of nanoparticles.

When proteins participate in the nanoparticle/biomolecule complex, a "protein corona" is formed[129]. For example, the human plasma system is abundant in proteins such as serum albumin, immunoglobulin G (IgG), and fibrinogen, which readily surround the surface of nanoparticles.[130, 131] Other proteins, such as apolipoprotein, may be much less abundant in the plasma system, but have higher affinities to the nanoparticle surface.[132] These proteins can, over time, competitively adsorb on the surface, displacing the already-adsorbed proteins.[133] It is important to note that abundant proteins with low affinities are not fully replaced by the proteins with higher affinities; they are also retained on the nanoparticle surface.[133]

During this competitive adsorption process, the corona proteins form two distinctive structures, "hard" and "soft" coronae (Figure 1.3). Proteins that are adsorbed tightly on the surface form the "hard" corona, while those that are loosely bound are called "soft". This identification method relies on the isolation of nanoparticle/protein particulates. It has been found that a few proteins (e.g. human serum albumin, apolipoprotein, and IgG) participate in the hard corona formation in human plasma system[134]. However, protein typing cannot distinguish between unbound proteins and soft corona, leaving the identification of soft corona proteins to a future challenge.

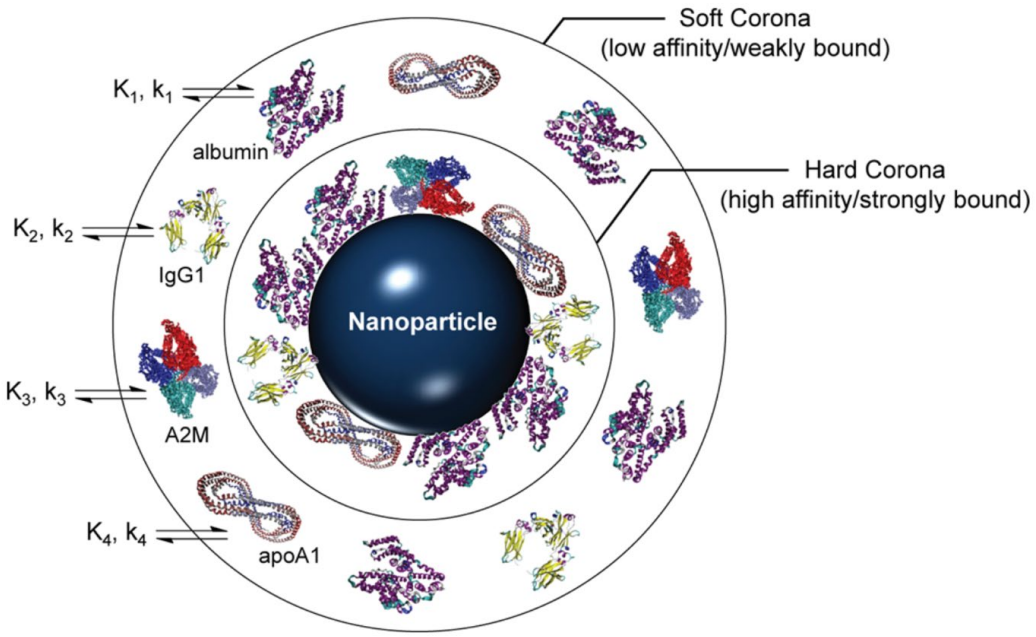

Figure 4.1. Schematic of protein corona formed around a nanoparticle, depicting two types of coronae, hard and soft. The figure is adopted with permission from an ACS publication and the original article can be found at https://pubs.acs.org/doi/10.1021/ar500190q.[135]

The presence of protein corona may or may not extend the lifetime of nanoparticles within biological organisms. Two classes of proteins play a crucial role, opsonins and dysopsonins. Opsonins act as an immunological barrier and are prone to cause phagocytosis due to their surface adsorption, thereby, shortening the lifetime of the external objects in the plasma system.[136] Major examples include immunoglobulins and their complementary proteins. Dysopsonins, on the other hand, are known to prolong their lifetime in the bloodstream. Albumin, the most abundant serum protein (constitutes 55% of plasma protein), belongs to this group, and is frequently found on the surface of the nanoparticles.[132, 137]

Corona formation can result in a loss of or alterations to the intrinsic functionality of proteins.[138, 139] Proteins participating in the hard corona, in particular, bind tightly to the nanoparticle surface, which can facilitate partial unfolding of their secondary structure.[138] Norde listed the thermodynamic forces driving the protein binding and protein conformational changes on solid surfaces: (1) electrostatic interactions between protein and solid surface; (2) dispersion force (van der Waals interactions), weak attractive force involving dipoles; and (3) enthalpic and entropic adjustment via conformational change responding to protein surface dehydration.[140] However, there are cases of stabilisation of the secondary structure upon protein corona formation.[141] There is also a case where the functionality of a soft corona protein was reported to be affected[139] even in the absence of structural alterations.

The corona structure also provides a platform for the corona proteins to modify their quaternary structure to undesirable forms.[142] Linse *et al.*[143] observed the enhanced formation of β_2-microglobulin oligomers, following their interaction with polymeric nanoparticles. Crucially, oligomeric states can then form amyloid-like protein aggregates, which are thought to be responsible for haemodialysis-associated amyloidosis (specifically for β_2-microglobulin).[144] The formation of oligomers alone can have a strong biological relevance, as in Alzheimer-related symptoms[145]. Conversely, nanoparticles can also inhibit the fibrillation of amyloid proteins[146]. These findings collectively highlight the case-by-case nature of the influence that nanoparticles have on protein quaternary structures.

Overall, the presence of protein corona is not necessarily deleterious. A benchmark study by Lesniak *et al.*[127] demonstrated a reduction in nanoparticle cellular adhesion and uptake due to the presence of protein corona. There were also reports that the nanoparticles with protein corona (compared to bare nanoparticles) weakened cytotoxicity,[147, 148] however, some report the opposite effect *in vitro*.[149] Since the cellular uptake is dependent on the types of proteins in corona, caution is advised in using the one-size-fit-all explanation for the role of protein corona.[150] Fleischer and Payne[126] showed the uptake mechanism is also affected by the secondary structure of the corona proteins, and is not influenced only by protein types. Notably, the above examples primarily use non-nanoplastics, and this case-by-case nature highlights the importance of testing out different combinations of nanoplastics (of different composition, size, and shape) and protein types.

4.1. Eco-corona around nanoplastics

Analogous to a protein corona, any molecules in the environment that participate in the corona structure satisfy the criteria for being an "eco-molecule" and for the resulting structure to be an environmental or eco-corona.[151] Eco-corona formation becomes a critical parameter in the predictive model, considering the ubiquity of nanoplastics in the environment. However, relevant studies have only recently appeared for microplastics[152]; few have considered this for nanoplastic research.

Research exploring the relevance of eco-corona (or often referred as adsorbed molecules) have targeted the molecules that are typically used in environmental toxicity research. The scopes of these studies are diverse and showed early evidences of; adsorption of organic pollutant on nanoplastics increases mobility (of pollutant molecules') in terrestrial environemnts[153, 154], microplastics facilitated bioaccumulation of pollutant molecules[155, 156], presence of eco-

corona affects the nanoplastic toxicity to fish[57], and synergetic toxicity with metal ions[157]. It has also been hypothesised that micro and nanoplastics could act a "Trojan horse" and transport the eco-toxic molecules to biological organisms (as seen in the case of bioaccumulation). The mechanism behind should be tackled at interfacial level. As demonstrated with proteins[133], the corona molecules undergo competitive adsorption and establish equilibrium with molecules in bulk solution. Same is applied for eco-corona, and therefore, the effect of eco-corona with further protein corona formation and chemical association becomes an important target of the future research.

Notably, the studies so far only assumed the classes of ecological molecules interacting with micro and nanoplastics for testing their ecological and biological impacts. To the best knowledge, these molecules constituting the eco-corona around micro and nanoplastics in nature have yet to be identified, and remain a critical challenge.

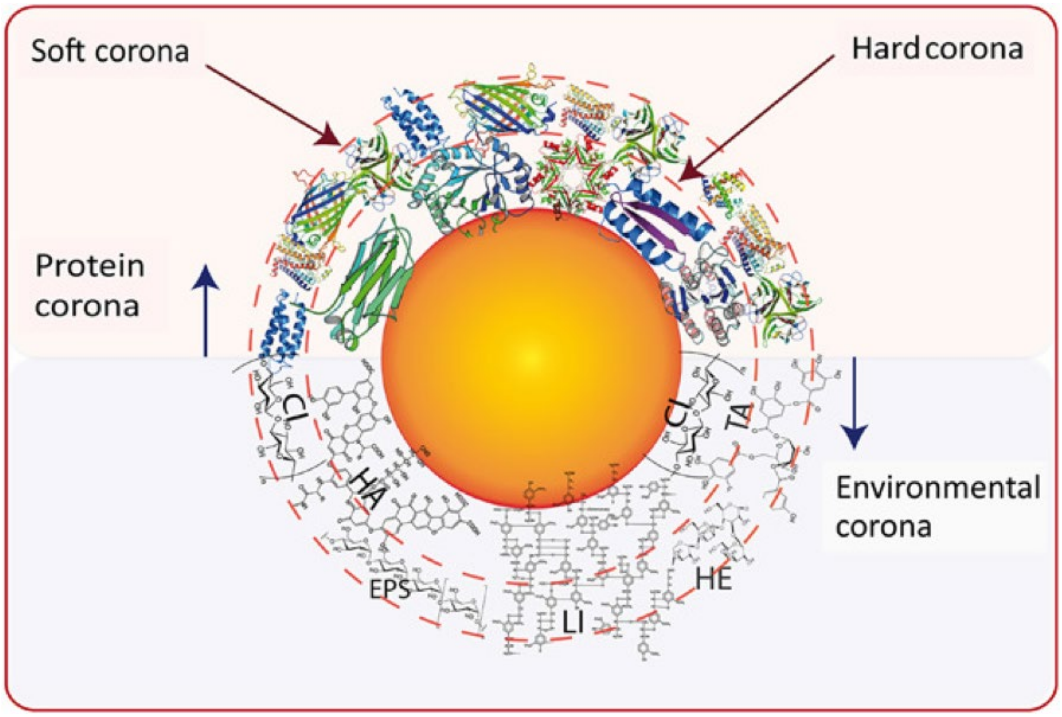

Figure 4.2. Comparative illustration of protein corona (top) and environmental or eco-corona (bottom) formed around a nanoparticle. Reprinted with permission from Pulido-Reyes et al. (https://setac.onlinelibrary.wiley.com/doi/full/10.1002/etc.3924) copyright (2017) John Wiley and Sons.

5. Opportunities for the interfacial scientist

Recalling the challenges of associating biological responses to nanomaterial properties, it is imperative for a predictive model (Figure 1.2) to understand the molecular and biological identities. However, due to the number of contributing factors involved, attempts to investigate this using *in vivo* and *in vitro* systems may impose many technical challenges. One approach is to simplify the bio-nano interface by creating model systems. This allows a systematic investigation of different parameters and resolution of molecular details at the interface. This approach has been implemented in the non-nanoplastic field, and has recently started to be adopted in nanoplastic research also.[124, 125, 158-160] We outline findings from both, non-nanoplastic and nanoplastic studies, that have focused on formation and structure of corona and its cellular interactions using model cellular membrane.

5.1. Uncovering corona formation and structure

A successful analytical approach to this complex challenge would identify the types of proteins in the hard corona *in vitro* and *in vivo*, both in steady state and resolved over time.[132] In contrast, model systems offer the possibility of further insight, including protein structural change, protein corona structure[124, 125], and adsorption behaviour.[161-163] For instance, the effect of corona formation (with varying particle size and electrostatic interactions) on participating protein secondary structure and binding constants was documented using spectroscopy techniques.[164] Similarly, a number of reports recorded a (partial) conformation change[124, 125, 138, 158], or, in some cases, stabilisation of the secondary structure[126]. Various factors are thought to contribute to this interaction; nanoparticle material, surface coating, coating density and pattern, particle size, shape, *etc*. To date, there is yet to be a unified theory connecting these physicochemical properties of nanoparticles, protein types, to these experimentally observed effects.

While the model system studies enable us to explore the physical parameters of individual proteins and nanoparticles, multi-component analysis is still a challenging task. Computational simulations have provided insight into the competitive adsorptions of proteins and nanoparticle behaviour, in multi-component systems.[133, 165, 166] Vilanova *et al*. [133] combined coarse grain modelling with binding constants for human serum albumin (HSA), transferrin, and fibrinogen to silica nanoparticles, experimentally obtained using fluorescence correlation spectroscopy.

Recently, computational modelling has been used for simulating nanoplastic interaction with proteins, predicting the affected structure as well as theorising the causes and effects.[158]

To carefully assess the relation between nanoplastic (or nanoparticle) properties and their toxicological profiles, the physicochemical properties of the complex formed with the protein corona (sometimes referred to as "biological identity"[134]) have to be considered[167], along with the particle characteristics. Thus, the structural evaluation of protein corona complexes have also been of considerable interest, and small-angle scattering techniques have supported this.[124, 125, 161, 168] This method (especially when used with contrast-matching techniques)[124, 125] provides an opportunity to understand individual components of a complex system when appropriate structural model is utilised. The structure of nanoplastic/protein corona complex and corona protein structure (soft and hard) was only recently evaluated using this technique (Figure 5).[124, 125]

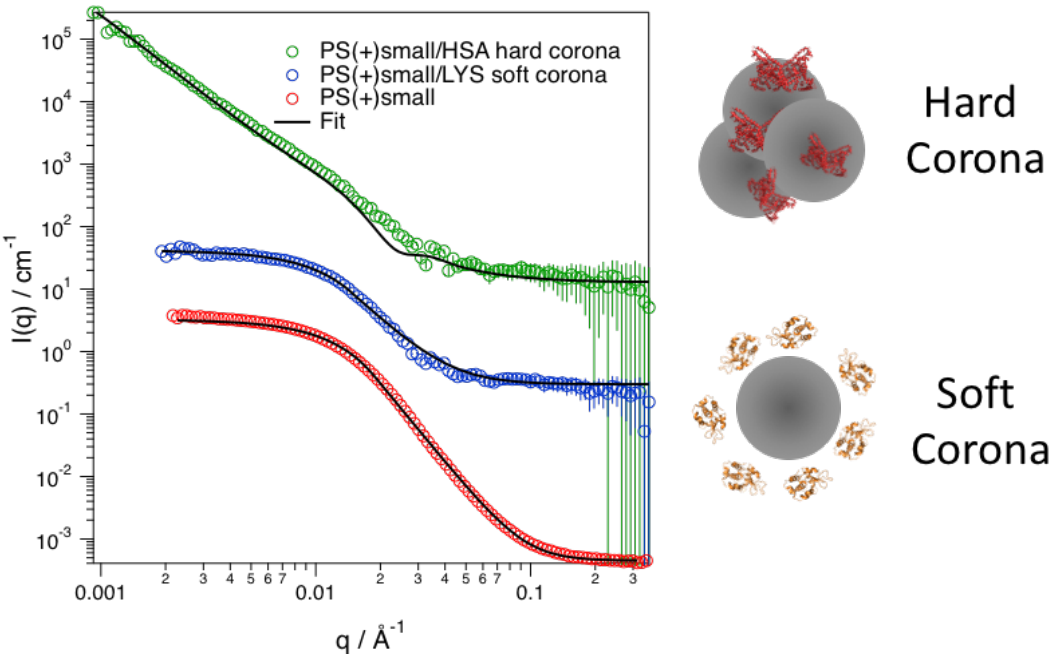

Figure 5.1. The structure of polystyrene nanoplastic complex with soft and hard protein corona, modelled based on the small-angle neutron scattering curves. The figure was adopted from ref 110 with permission from the AIP publishing.

It is worthwhile noting that studies have mainly documented the interaction of nanoparticles with proteins. However, the nanoplastic exposure in humans and other organisms would inevitably occur in environmental matrices which contain a molecular cocktail and form an eco-corona. To our knowledge, few studies have shown the significance of eco-corona.[169-171] The types of molecules found on nanoplastics from the environment, eco-corona structure, their influence on further protein corona formation, and subsequent biological interactions are yet to

investigated– there is still a considerable knowledge void. We believe that the research articles introduced in this review embody methodologies worth exploring.

5.2. Implications of nanoplastics and corona-complex

Ignorance about biological identities and their biological impact remain one of the challenges to complete the scheme of the predictive model (Figure 4.1). Chiefly, the question of how and which components of nanoplastic/corona complex affects further interactions with biological entities such as cells, are largely unaddressed. Contributory factors could include nanoplastic material, protein type found in soft and hard corona, morphology of nanoplastic/corona complex, structure of participating proteins (from secondary to quaternary). These factors can be tested by *in vitro* experiments, which investigate detailed cellular interactions and their responses in the form of cellular uptake, localisation, cytotoxicity, oxidative stress, chromosomal aberration, *etc*. While these experiments yield insightful information, the mechanism relating to the interactions with individual components remain open to question. In the past, model systems such as lipid bilayers have demonstrated their use for studying interactions with other biologically active molecules such as proteins, peptides and drug candidate molecules, as well as the lipid bilayer undergone oxidative stress [172-176]. Furthermore, the sparsely-tethered lipid bilayer in particular has shown to be a better mimicry of the natural cellular membrane – effectively being used to study the above mentioned aspects.[172, 176-180]

A model lipid bilayer has also been applied to study the cellular interactions with nanoparticle systems.[181-186] These studies demonstrate that the aforementioned physicochemical properties of nanoparticles can affect the structural integrity and membrane fluidity, both of which are vital in maintaining cellular functions. In some cases, these bilayer properties were sensitive to surface patterning of nanoparticles.[123, 187] While many studies focused on bare nanoparticles, few have shed light on the nanoparticle/corona complex[188], in fact, the number of studies are even more limited than for nanoplastic/corona complex.[128]

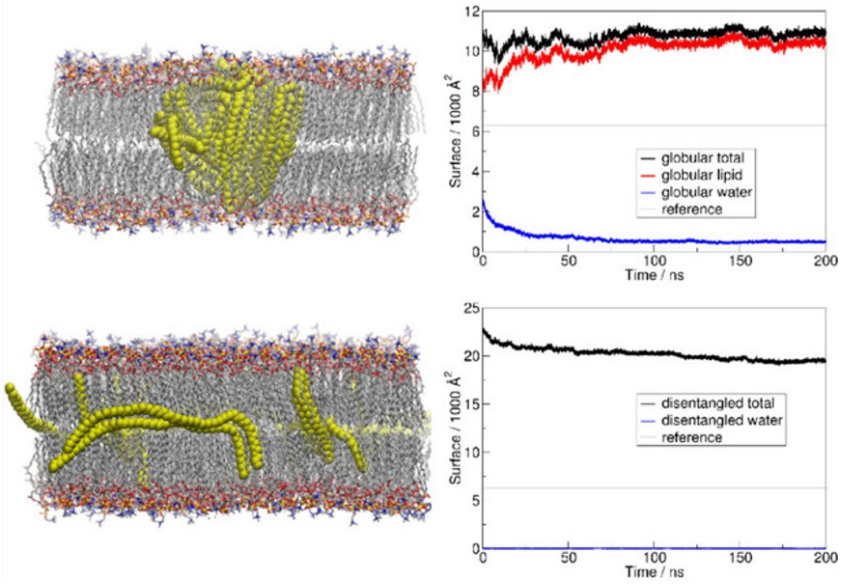

Figure 5.2. Simulated interactions between model phospholipid bilayer and polyethylene nanoparticles of different shapes. The figure was reproduced from Ref 138 with permission from European Chemical Society Publishing.

Proteins participating in the corona formation can drastically change cellular interactions (compared to nanoparticles), although the cause is open for debate – whether it is attributed to protein structural change, formation of new morphologies (nanoparticle/corona complex), or a combination of the two. As discussed, the participating corona proteins can lose their structural integrity which may disrupt the lipid bilayer upon contact (while their native form is membrane-inert).[189] To complicate matters, it is also affected by the nanoparticle physicochemical properties and the protein types, all of which need to be carefully assessed. A lack of evidence and variables explored (particularly with nanoplastics) prevents further assessment. Studies found in the literature have been limited to using polystyrene as a model nanoplastic and commonly found proteins (e.g. serum albumin and lysozyme). Evidence suggests that less abundant proteins constitute the protein corona[132], and types of corona proteins (or the combination of which) affect their cellular response.[190] Based on the methodologies employed by the studies highlighted here, future studies should consider the usage of other polymer material and more specialised proteins.

6. Summary and future outlook

The present work reviewed recent findings relating to the potential impact of nanoplastics on biological organisms (i.e. microbial, aquatic, and implications for humans). Collectively, these studies support the potential of nanoplastics to disrupt the ecological function of biofilms, cause adverse effects in aquatic organisms, and to bioaccumulate. There is no evidence yet that shows

major nanoplastic uptake by humans, however this should not be considered final. The potential effects for humans are largely discussed on the basis of *in vitro* experiments and theories extrapolated from non-plastic nanoparticles. We highlight an approach taken in nanotoxicology, that attempts to establish a link between physicochemical properties of nanoparticles and their impact (e.g. physiological effects) on the basis of chemistry and biology. This mechanistic approach allows for future decision-making to mitigate the harm caused by nanoplastics as it can be tailored to the level of risk predicted. We highlight the main gaps in the nanoplastic field: 1. Lack of understanding behind the influence of physicochemical properties (plastic types, size, shape, *etc*) of nanoplastics on corona formation (both protein and eco-corona), 2. The impact of eco-corona on protein corona formation, 3. The biological impact of eco-corona and protein corona around nanoplastics (from cellular to model organisms), 4. Identification of molecules participating in eco-corona in nature. While these questions can be addressed in part via *in vitro* experiments, molecular details are difficult to obtain. These are important parameters which can attribute the observed biological consequences to the nanoplastic (and nanoplastic/corona complex) properties. The methodologies employed in interface science are particularly useful in addressing these questions, from understanding the formation and structure of protein corona in nanoplastic property and a protein-type-dependent manner to resolving the lipid bilayer interaction with molecular resolution. Nanoplastic-specific studies attempting to explore these points are scarce and leaves significant opportunities for future research.

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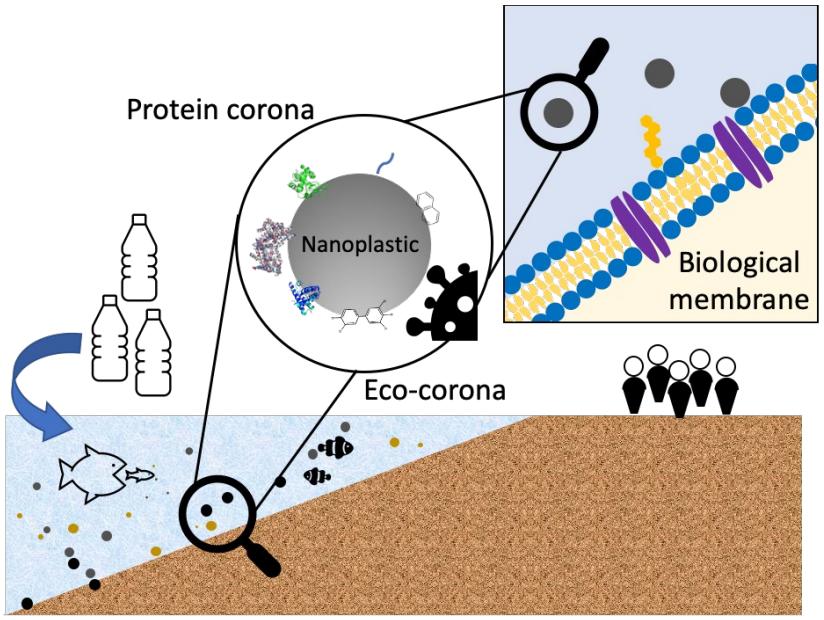
501 Abstract Figure



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504 ## 7. References

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