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REVIEW

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The role of patient-specific variables in protein corona formation and therapeutic efficacy in nanomedicine

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Abstract

Despite their potential, the adoption of nanotechnology in therapeutics remains limited, with only around eighty nanomedicines approved in the past 30 years. This disparity is partly due to the "one-size-fits-all" approach in medical design, which often overlooks patient-specific variables such as biological sex, genetic ancestry, disease state, environment, and age that influence nanoparticle behavior. Nanoparticles (NPs) must be transported through systemic, microenvironmental, and cellular barriers that vary across heterogeneous patient populations. Key patient-dependent properties impacting NP delivery include blood flow rates, body fat distribution, reproductive organ vascularization, hormone and protein levels, immune responses, and chromosomal differences. Understanding these variables is crucial for developing effective, patient-specific nanotechnologies. The formation of a protein corona around NPs upon exposure to biological fluids significantly alters NP properties, affecting biodistribution, pharmacokinetics, cytotoxicity, and organ targeting. The dynamics of the protein corona, such as time-dependent composition and formation of soft and hard coronas, depend on NP characteristics and patient-specific serum components. This review highlights the importance of understanding protein corona formation across different patient backgrounds and its implications for NP design, including sex, ancestry, age, environment, and disease state. By exploring these variables, we aim to advance the development of personalized nanomedicine, improving therapeutic efficacy and patient outcomes.

Keywords Protein corona, Nanomedicine, Nanotechnology, Nanoparticles

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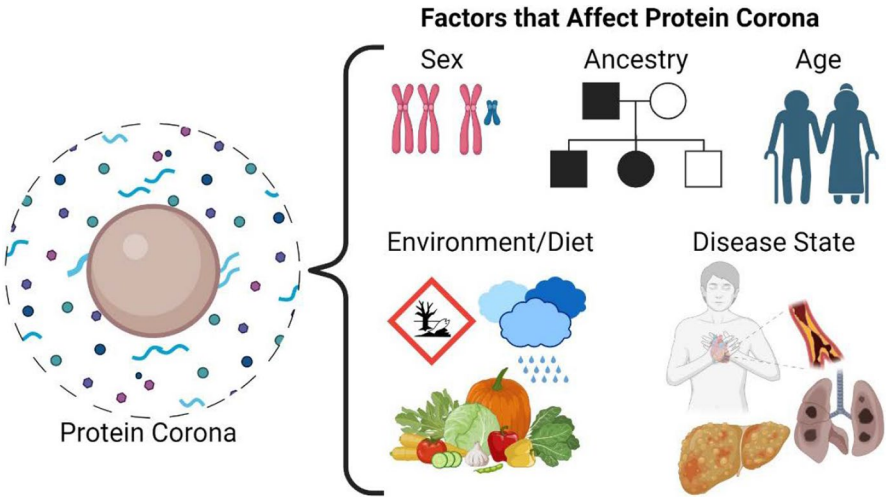
Graphical Abstract



Background

Nanoparticles (NPs) have great potential to revolutionize the field of drug delivery, as they can provide controlled release of therapeutics, overcoming biological barriers to transport, targeting delivery to specific areas of the body, and protecting the therapeutic from premature degradation [1, 2]. Due to these advantages, NPs improve both the safety and efficacy of therapeutics, which leaves a conundrum as to why more therapeutics are not being delivered via nanotechnology. First approved by the FDA in 1995, Doxil is a liposomal-based NP to deliver doxorubicin [3]. However, the approved nanotechnologies since then have been limited, with around 80 nanomedicines total approved in the past 30 years compared to around 50 new drugs approved each year. Only around 14% of nanotechnology phase 3 trials for cancer are successfully introduced into clinic practice [4]. This is in part due to our tendency as engineers to approach medical design from a "one-size-fits-all" perspective [1], while neglecting patient-specific variables that could affect the pharmacokinetics and pharmacodynamics of NPs

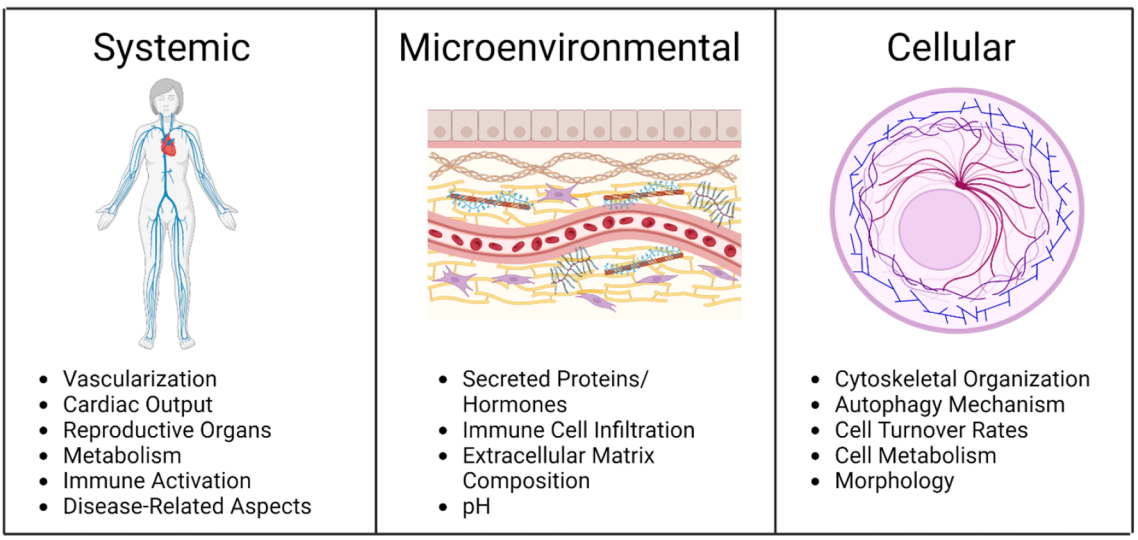


Fig. 1 Patient-dependent properties at the systemic, microenvironmental, and cellular levels that influence NP delivery and efficacy. Created with BioRender.com

such as biological sex, genetic ancestry, disease-state, environment, and age.

NPs must traverse multi-layered barriers to deliver therapeutics to their target, at the systemic, microenvironmental, and cellular levels; of course, these barriers vary across heterogenous patient populations. Patient-dependent properties that influence NP delivery and efficacy include blood flow rates, body fat distributions, reproductive organs (and respective vascularization at the reproductive organs), hormones and other proteins, immune system responses and phagocytic clearance, and chromosomal differences that may cause cellular level differences (e.g. actin filament density); some of these variables that may cause differences to arise in NP delivery are shown in Fig. 1. Considering all these variables, it is important to determine which are driving forces of NP behavior, and how we can utilize these variables to our advantage and create nanotechnologies for specific subsets of patients.

Three of the most significant properties of the NP that determine its distribution and interactions with its target are the NP size, shape, and surface charge. Once in contact with biological fluid, protein aggregates, termed "protein corona", adsorb rapidly around the surface of the NP. Protein adsorption to the NP surface is a kinetic and thermodynamic function of the proteins and NP properties. Importantly, the protein corona will determine the new size, shape and charge of the NP, as well as its propensity toward aggregation, as shown in Fig. 2. These characteristics affect the behavior of the NP with respect to biodistribution, pharmacokinetics, cytotoxicity, and organ trafficking/targeting [5]. This in turn determines subsequent effects on the cell and organ function [6]. In particular, the protein corona will be responsible for controlling which cellular receptors the NP binds to, cellular internalization pathways, and immune response [7]. Additionally, the protein corona can also have an effect on drug release kinetics for NPs that are being used to deliver therapeutics at specific rates [8].

The composition of the protein corona is governed by both protein-NP and protein–protein interactions, influenced by the original NP's characteristics as well as the protein and other analytes present and their concentrations in the patient's biological serum [6]. The protein

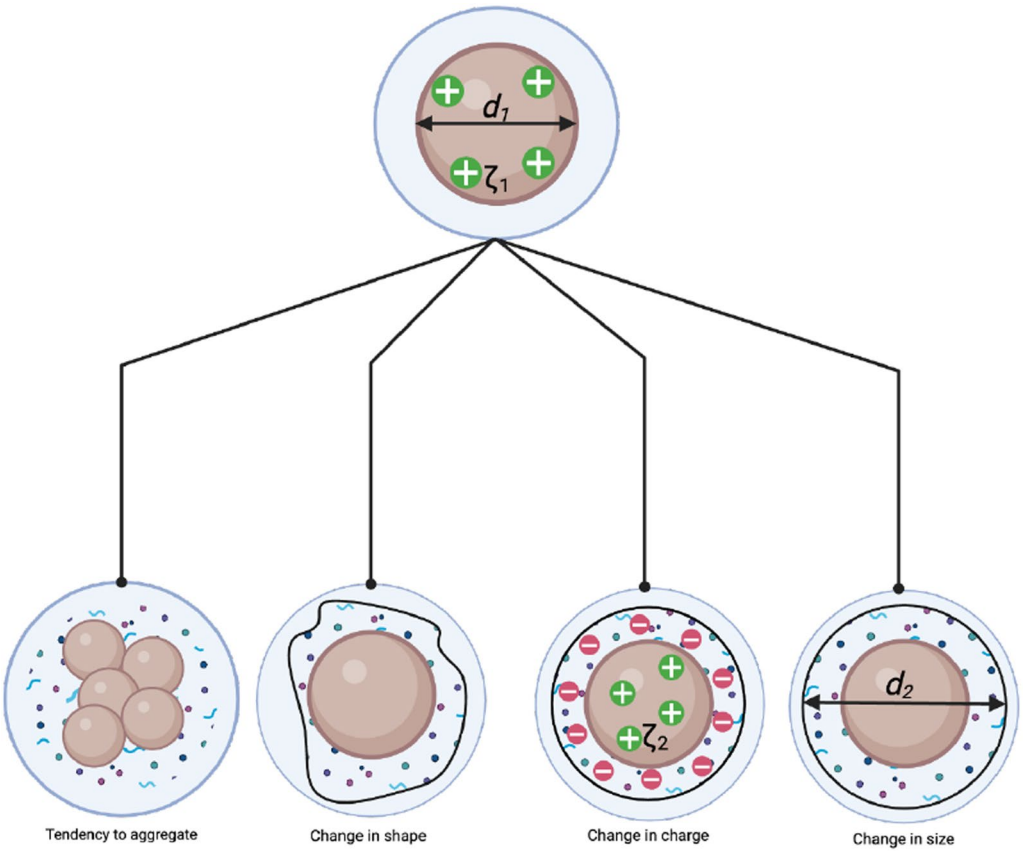


Fig. 2 Exposure of a NP to biological fluids causes a protein corona formation that can alter key properties of the NP: aggregation tendency, shape, charge, and size. Created with BioRender.com

corona is also affected by exposure time to the biological serum, as dynamics of surface adsorption and binding kinetics of various proteins will vary transiently. It has been shown in the literature that early protein adsorption to NPs in blood is often dominated by hydrophobic proteins such as apolipoproteins that are in higher concentrations in the blood, which become replaced over time with proteins that are less abundant but have a higher affinity for the NP surface [6].

As engineers, we need to seek to fully understand the protein corona that forms on the surface of NPs and other biomaterials, and how this will vary depending on both the NP and patient properties. In regards to NP properties, protein folding on the NP surface has been shown to be influenced by the NP's charge. For example, one study showed that when bovine serum albumin (BSA) was adsorbed to a cationic NP, the structure of the BSA was altered, but it was not altered when adsorbed to an anionic NP [7]. This alteration in structure led to cellular binding to scavenger receptors for the cationic NPs, where the anionic NPs bound to albumin receptors on the cell's surface. This finding shows that even particles with identical protein coronas can have different responses in vivo due to the protein structure in the protein corona, which is dependent on the properties of the NP.

In practice, protein coronas have diverse effects on NP behavior which affects therapeutic efficacy. A study by Pourjavadi et al. tested the effects of protein corona on doxorubicin release from magnetic mesoporous silica NPs coated with poly(ethylene glycol) (PEG) [9]. PEGylated NPs showed decreased protein adsorption than non-PEGylated NPs. As a result of reduced protein corona, greater drug release was demonstrated for PEGylated NPs than uncoated NPs [9]. This is important as tuning release properties is critical for therapeutic efficacy. Moreover, the composition of the protein corona can also influence efficacy. Vincent et al. used poly(ethylene glycol)-b-poly(propylene sulfide) (PEG-b-PPS) polymersomes with various chemistries including methoxy, hydroxyl, and phosphate end groups to explore modulation of the protein corona [10]. On the cellular level, albumin was found in abundance in the protein coronas of all formulations and accounted for increased uptake in macrophages.

Protein corona has also been found to directly affect therapeutic efficacy in the clinical setting. Onpattro, a lipid NP siRNA therapy, relies on the formation of protein corona containing apolipoprotein E (ApoE) for hepatocyte-specific targeting. This is accomplished by using DMG-PEG, a C14 PEG-lipid, which allows it to desorb from the particle and exchange with ApoE [11]. This gives the NP targeting capabilities to hepatocytes. Importantly, the DMG-PEG allows desorption from the particle whereas a lipid with a longer acyl chain (e.g. DSPE, DOPE) would not allow for such exchange as they stably associate with the NP. Without the presence of an ApoE-containing protein corona or DMG-PEG on Onpattro, this exchange does not occur which has been shown to have decreased hepatic uptake [12, 13].

Of course, patients from different backgrounds stratified by sex, ancestry, age, environment, and disease, will also exhibit variations in the protein corona when interacting with the same NP. In oncology, patient stratification based on biomarkers and companion diagnostics is standard practice for achieving positive outcomes [14]. By studying stratified patient groups at various stages of our research, we can gain a deeper understanding of the factors driving differences among patients, paving the way for a more accessible approach to personalized medicine. This review aims to demonstrate how varying patient characteristics influence the formation of the protein corona and how this knowledge can be used to better engineer NPs for specific patient groups. While sex and disease state are the most commonly considered variables, we must also consider other patient characteristics that may contribute to these differences. In this review, we focus on the protein corona that forms upon contact with blood; however, alternative administration routes, such as oral or vaginal delivery, will also form a unique protein corona based on the biological fluids in these environments. We explore previous research on NPs and protein corona development based on sex, ancestry, age, environment, and disease state, and discuss future directions in this field. Our goal is to enhance our understanding of these variables and their impact on the design of patient-specific NPs.

Main text

The effects of biological sex on the protein corona

Difference between sex and gender

Sex and gender are often used synonymously; however, it is important to make distinctions between these two terms as they are both known to contribute to health from etiology to outcomes [15, 16]. On the most basic level, sex is a biological factor related to genetics, physiology, and anatomy and can be attributed to differences that arise on cellular and molecular levels [5]. Biological differences can include the presence/absence of the Y chromosome, bone shape and density, metabolic regulation mechanisms, and presence/absence of reproductive organs. Gender is a social construct and refers to an individual's identity, expression, and presentation as man, woman, or non-binary [17]. Gender is shaped by sociocultural factors such as prescribed gender roles, power dynamics, and relationships. The interaction of gender

and medicine can be seen through help-seeking behavior, disease perception, decision making, and use of health care [18]. Conflation of sex and gender, both inadvertently and intentionally, is more harmful than neutral to health research. Such aggregation leads to incomplete analysis and can lead to adverse health outcomes and gaps in innovative medical treatment and policy [19, 20]. While there are arguable differences between sex and gender, it is important to note that they are both fluid. Sex is on a spectrum and can be culturally influenced as well. Variations in chromosomes, hormone levels, and reproductive organs can result in more than two sexes [17]. Similarly, the concept of gender varies as a result of institutional and cultural change [17].

Nanotechnology and sex

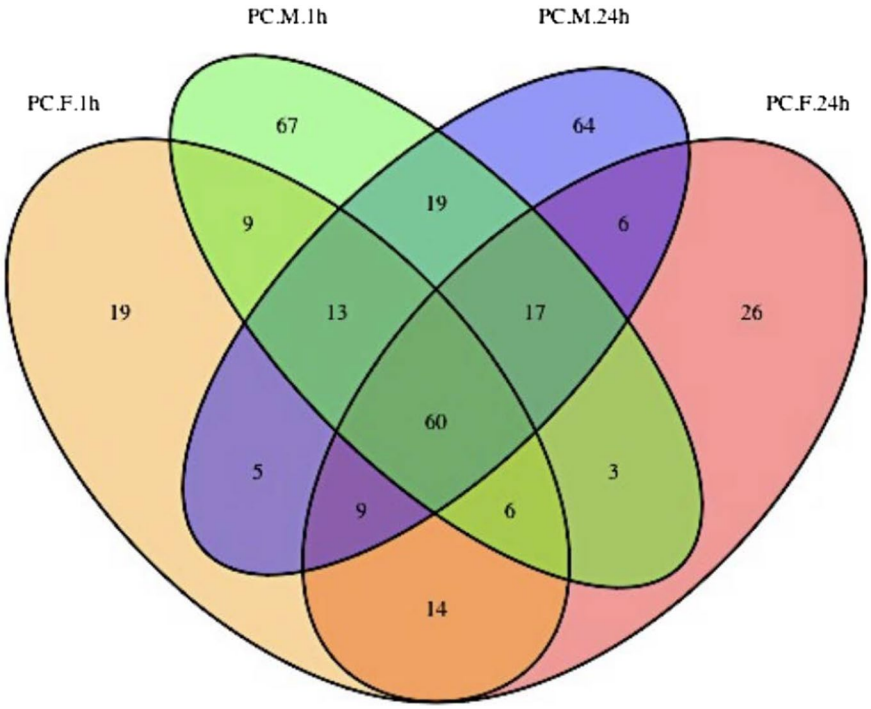
Sex-based differences arise on a spectrum from variables including differences between the X and Y chromosome, X-inactivation, sex steroid hormones, reproductive organs, fat distributions, and more. In the past, these differences have led to different drug metabolism rates, and lack of inclusion in drug trials led to higher hospitalization rates and adverse effects in women [21]. Investigational New Drug Applications often neglect sex as a variable, but the ones that include it have shown up to 40% differences in pharmacokinetics [22]. These differences include properties such as drug absorption, metabolism, distribution, and excretion.

These differences can also affect the performance of nanotechnology. Researchers have begun to investigate sex-based differences which has revealed variability between sex in the biodistribution of NPs in different tissues. For example, females have greater accumulation of NPs in the kidneys as compared to males. Conversely, males have greater accumulation of NPs in the liver as compared to females [5]. Besides biodistribution differences, therapeutic effects between sexes can differ as well. An inflammation model in mice using macrophage-targeted nanotheranostics revealed sex-based differences in COX-2 inhibition, an enzyme driving inflammation, indicating greater therapeutic success in males versus females [23]. Furthermore, in an in utero study, only hypoxic female mice responded positively to MitoQ NP treatment where pro-angiogenic factor *Vegfa* and growth factor *Igf2* expression increased [24]. Possible explanations for differences in biodistribution and therapeutic efficacy can be delineated through natural physiological processes. For instance, the menstrual cycle effects have also been shown to affect both biodistribution of NPs and therapeutic efficacy for applications in ovarian and breast cancer in a study by Poley et al. They found that gadolinium NP accumulation was the highest in the ovaries and uterus during ovulation. Based on this finding, they delivered therapeutic PEGylated liposomal doxorubicin NPs, it was found that efficacy was highest during ovulation when treating ovarian cancer. Simultaneously, when mice were treated for breast cancer during the same period, NPs continued to accumulate in the reproductive system which led to decreased efficacy in treatment [25]. Differences in biodistributions seen over the menstrual cycle in this study can be explained from an anatomical perspective; increased vascularity at different stages in the menstrual cycle can lead to differential drug trafficking causing some organs to accumulate therapeutics. Glaringly, males do not have this physiology. Therefore, it is important that NPs designed for females be modified to account for these differences. To further improve NP formulations, investigating protein coronas could provide a starting point into how performance of NPs differ with respect to biological sex.

Protein corona and sex

Sex differences in serums are well-documented in medical literature. These differences include statistically significant variability in concentrations as well as presence/absence of proteins and other analytes in metabolic activity [26–31]. As a result of sex-linked differences in serum compositions, sex has been implied as a critical factor that affects NP performance with respect to sex-specific protein coronas [5, 32, 33]. Sex-specific protein coronas can affect the performance of an NP since differential proteins are recruited which can affect its size, surface charge, shape, and chemistry. The modification of an NPs physicochemical characteristics, in turn, affects the NP-cell interactions [34].

Sex-based differences in protein corona formation in non-human species Most research in investigating sex-based differences in protein corona has been more exclusive to non-human species. In a pioneering study by Hayashi et al., 70 nm SiO_2 NPs were used to test whether biological identity from protein corona affected uptake into immune cells from zebrafish. In all the serums tested, apolipoprotein A-I was detected in the hard protein corona of the NPs validating the plausibility of this study [35]. Vitellogenin and fetuin were found in abundance in NPs soaked in female and male plasma, respectively, revealing sex-specific biological identity in the protein corona. Vitellogenin is an egg precursor protein present in zebrafish oviparous reproductive systems which accounts for the difference in protein aggregation onto the NP surface. When testing NPs pre-soaked in either male or female serum, it was found that those soaked in female serum were preferentially uptaken into both myeloid and lymphoid cells isolated from whole kidney marrow cells. However, it should be noted here that the



Summary of Key Findings		
Criteria	Groups	Number of Proteins
Unique proteins	F, 1h	135
	F, 24h	147
	M, 1h	194
	M, 24h	193
Shared proteins	All	18
	M, F	40
	F	89
	M	109

Fig. 3 Proportion of shared proteins in protein coronas between male (M) and female (F) fish and incubation time (1 h or 24 h) along with a summary of key findings from Gao et al. [6]. Reprinted with permission from Gao et al. [6] (Copyright 2017 American Chemical Society)

authors did not report the sex of the cells which were used for these studies, and this could be a relevant factor as well. Sexual dimorphism in cells should be a variable that is tested or controlled for since there is well-documented sex-dependent gene expression and differential cellular responses due to hormonal exposure [36–40]. While the biological mechanism in preferential uptake requires further investigation, the authors offer a suggestion that proteins on the surface of the NP prompt innate immune responses since they may have aided in NP recognition [35].

Based on this study, an extension of this study by Gao et al. showed similar results overall as Hayashi et al. in smallmouth bass using polyvinylpyrrolidone-coated silver NPs (PVP-AgNPs) [6]. 50 nm PVP-AgNPs were soaked in female and male plasma and showed an increase in size by about 8 nm. Zeta potential measurements showed greater instability and changes in PVP-AgNPs in male plasma versus female plasma. Furthermore, less than 40% of the proteins in the protein corona were common between sex. Here, both zona pellucida (ZP) and vitellogenin, proteins taken up in developing follicles, accumulated in the protein corona of PVP-AgNPs. With vitellogenin and ZP accumulating preferentially, the combination with PVP-AgNPs can cause localization to the ovaries in fish. This study also

showed that proteins commonly associated with the immune system, notably immunoglobulins and complement proteins, accumulated more abundantly on the surface of PVP-AgNPs [6]. Previous work using PVP-AgNPs in fish have shown adverse effects in offspring and follicular development. Based on this work, NPs could be more toxic to female fish due to potential accumulation in the ovaries, an organ that is absent in males. Further experiments in delineating NP organ accumulation would definitely be an interesting extension of this study. Another interesting extension could be extracting blood from specific organs, identifying proteins specific to each sex, and testing their effect on NP accumulation in the organ. Figure 3 shows the amount of shared protein in the corona when PVP-AgNPs were incubated in male or female for 1 h or 24 h.

In a study by Ashkarran et al., silica NPs (SiNPs) of varying particle size and porosity were soaked in mice plasma to test sex-based differences in protein corona recruitment [41]. Initial characterization of the NPs showed that the diameter of the all NPs increased by 8–35 nm and zeta potential slightly decreased (more positive) indicating slightly lower colloidal stability. In mesoporous SiNPs, NPs containing numerous small pores, at approximately 100 nm size, 17 proteins were found to be significantly greater in SiNPs soaked in male plasma versus female plasma. Resistin, a protein involved in glucose uptake suppression and inflammation, was the protein found to be significantly higher in male protein corona. Other identified significantly different proteins include complement factors C8a, C8b, and C8g which are proteins responsible for innate and adaptive immunity. In Stöber SiNPs (non-mesoporous) at 100 nm size, male urinary protein 1, a protein binding pheromones from male urine, was found to be significantly greater in SiNPs soaked in male plasma versus female plasma validating the approach of this study. Here, proteins related to inflammation and tumorigenicity were found to be significantly greater in male soaked SiNPs than female soaked SiNPs including Krt16, Ig-heavy chain V region MOPC 173, and carboxylesterase ID. Similar results were observed in Stöber SiNPs (non-mesoporous) at 50 nm size where Rab27b, Ig-heavy chain V region 5–84, H4c1, and Ig-heavy chain V region MOPC 173 were all found to be significantly in male soaked SiNPs than female soaked SiNPs. Table 1 shows a summary of proteins found at significant levels in the protein corona of NPs with varying porosity and size.

Based on the differential proteins adsorbed onto the mesoporous and the Stober 100 nm and 50 nm particles, it was shown that size and porosity play a role in protein corona formation [41]. Notably, in the mesoporous SiNPs' protein corona, there were significantly more unique proteins versus the non-mesoporous SiNPs. Porosity increases the amount of surface area relative to volume allowing for increased protein absorption onto the NP. An extension of this work on size could be the addition of a polymer to "standardize" protein corona recruitment to measure the same identity and similar concentrations of protein between male and female plasma. Additionally, protein coronas formed from male plasma showed significantly greater protein recruitment than female plasma implying that biosensing capacity was greater for the males versus females. An interesting extension of this work could be to use the SiNPs to test protein recruitment for mice that have a known disease. This could reveal and quantify diagnostic efficacy for nanotechnologies as well as clarify any sex-based differences in clinical biomarkers that may arise in practical use. Table 2 shows a summary of sex-based differences in NP parameters in different species.

Table 1 Proteins measured in protein coronas at significant difference based on sex for each NP type from Ashkarran et al. [41]. Reprinted from Ashkarran et al. [41], under creative commons license

NP Type	Protein
Mesoporous 100	Retinin
	Complement C8 α chain
	Complement C8 β chain
	14-3-3 protein eta
	60S ribosomal protein L12
	Carboxypeptidase N catalytic chain
	Ig lamda-2 chain C region
	Ankyrin-1
	Heterogeneous nuclear ribonucleoprotein Q
	T-complex protein 1 subunit zeta
	BPI fold-containing family A member 2
	GTP-binding nuclear protein Ran, testis specific isoform
	Dynamin-1
	Eukaryotic translation initiation factor 2A
	Complement C8 γ chain
	40S ribosomal protein S19
	Cathelicidin antimicrobial peptide
Stöber 100	Krt16
	Ig heavy-chain V region MOPC 173
	Mup1
	Ces1d
Stöber 50	Rab27b
	Ig heavy-chain V region5-84
	H4c1
	Ig heavy-chain V region MOPC 173

Table 2 Summary of parameters from literature using non-human species for sex-based difference studies

	Reference		
Property	**Hayashi et al. [35]**	**Gao et al. [6]**	**Ashkarran et al. [41]**
NP material	SiO_2	PVP-AgNP	Silica
Species	Zebrafish	Smallmouth Bass	Mice
Avg. NP size before plasma (nm)	73.6	49.4–52.6	S50[1]: 48.4; S100[2]: 98.7; M100[3]: 105.7
Avg. NP size after plasma (nm)	112.0 (F); 103.7 (M); 106.3 (Mix)	57.3–60.8 (F); 56.1–58 (M)	S50 (M): 56; S50 (F): 62.2; S100 (M): 135.3; S100 (F): 103.1; M100 (M): 108.7; M100 (F): 122.4
Avg. NP zeta potential before plasma (-mV)	42.7	30.2–34.7	S50: 15.0; S100: 14.3; M100: 10.5
Avg. NP zeta potential after plasma (-mV)	31.6 (F); 32.4 (M); 30.9 (Mix)	20.1–20.7 (F); 16.6–17.2 (M)	S50 (M): 14.6; S50 (F): 10.0; S100 (M): 9.2; S100 (F): 10.3; M100 (M): 14.3; M100 (F): 11.3
NP porosity	N/A	N/A	Mesoporous particle
Model (in vitro, in vivo, or ex vivo)	Ex vivo, in vitro	Ex vivo	Ex vivo
Plasma type	Blood plasma, whole kidney marrow	Blood plasma	Blood plasma
Sex-differences observed	NPs soaked in female plasma were preferentially taken up by myeloid and lymphoid cells; Vitellogenin found in abundance in female protein corona; Fetuin found in abundance in male protein corona	Significant differences in zeta potential after soaked in plasma; vitellogenin and ZP found in protein coronas from female plasma; fewer than 40% proteins common between sexes	Inflammatory and tumorigenic proteins found in greater amounts in male protein coronas; Resistin (inflammatory) had the largest significant difference in male protein corona; Male protein corona has significantly greater protein recruitment
Possible mechanisms	NP-bound endogenous proteins could trigger innate immunity	Vitellogenin and ZP form on the "soft" corona and are presented to follicular cells for uptake	Differential enrichment in the corona due to the presence/absence of proteins in a certain sex influenced by binding kinetics

[1] Denotes Stöber 50 nm SiNPs, [2]Denotes Stöber 100 nm SiNPs, [3]Denotes Mesoporous 100 nm SiNPs

While there has been progress in the field of sex-based nanomedicine in non-human species, it would be interesting to see experiments using primates to identify proteins more applicable to humans. Testing nanotechnologies in harvested blood/tissue samples from specific primate organs, specifically the GI tract, liver, and kidneys, could provide valuable insight into what proteins promote clearance, uptake, cytotoxicity, and immune responses in each of these organs so that scientists can improve formulations to yield better clinical trial results.

Sex-based differences in protein corona formation in humans There is limited research discussing biological and molecular mechanisms of sex-based differences in humans, as shown in Table 3. However, a notable study by Vulpis et al. revealed sex may be an important factor in nanomedicine [42]. In their study, DOTAP lipid NP uptake was tested in six distinct immune cells differentiated by sex. It was found that uptake in natural killer (NK) cells, lymphocyte cells critical in innate immunity and immune response regulation, was significantly greater in male cells. These results suggest that the difference in uptake between the male and female NK cells lead to disparate immune system performance. The researchers then repeated the uptake studies by pre-treating the NPs with human plasma to test the effects of protein corona. By pre-treating with human plasma, cell-NP interactions are able to be controlled better. The pre-treatment of the NPs eliminated the sex differences shown in NK cell uptake and dramatically reduced uptake in all immune cells tested. Hence, these results suggest that pre-forming a protein corona can help to mitigate and standardize immune responses in patients regardless of sex [42]. This study introduces a unique strategy to eliminate sex-based differences in nanomedicine; a standard "cocktail" of human plasma could be developed to attenuate the effects of sex in nanomedicine. To expand upon this study, researchers could consider how hormones and other sexual dimorphisms could affect uptake and immune response.

However, a challenge faced by researchers attempting to evaluate biological sex in nanomedicine is interference from hormonal variations. In a study by Serpooshan et al., human amniotic stem cells (hAMSCs) were used to circumvent this issue, as hAMSCs represent one of the earliest stages of somatic stem cells since they have sexual dimorphism without the initiation of hormonal changes [34]. Hence, the differences in NP performance should relate to genetic and structural dimorphisms. In their study, cytokine release in hAMSCs was compared before treatment with quantum dots. Prior to treatment, it was found that 14 cytokines were found to be at significantly higher levels in male hAMSCs versus their female counterparts. With respect to cytokines, NP-cell behavior could be altered with respect to immune response; the recruitment of certain cytokines could induce an immune response or trigger pathways in one sex versus the another.

Sex-specific hAMSCs and primary fibroblasts were then treated with quantum dots (QDs) controlled for size and surface chemistry. Flow cytometry results revealed greater accumulation of QDs in female hAMSCs as opposed to male hAMSCs. To partially explain this difference, QDs were incubated with serum and the zeta potential was measured. The initial QDs' surface charge was -22 ± 1 mV. After exposure to serum, the QDs' surface charges were -15 ± 1 and -11 ± 1 mV for female and male serum, respectively. A more negative zeta potential indicates greater colloidal stability

Table 3 Summary of parameters from literature using human species for sex-based difference studies

	Reference	
Property	**Vulpis et al. [42]**	**Serpooshan et al. [34]**
NP material	DOTAP	Quantum dots (Qtracker 525)
Avg. NP size before plasma (nm)	~140	N/A
Avg. NP size after plasma (nm)	~215	N/A
Avg. NP zeta potential before plasma (-mV)	~60	22
Avg. NP zeta potential after plasma (-mV)	~−20	15 (F); 11 (M)
Model (in vitro, in vivo, or ex vivo)	Ex vivo, in vitro	In vitro
Plasma type	Blood plasma	hAMSC supernatant
Sex-differences observed	NP uptake in NK cells is significantly greater in male cells; Soaking NP in plasma eliminated NK uptake sex-difference	Better colloidal stability in female-soaked QDs; Greater and faster QD uptake in male fibroblasts; Greater uptake in female hAMSCs; Cytokines found in greater amounts in male hAMSCs
Possible mechanisms	Females have less NK cells due to aging and menopause	Male cells have less actin filaments and more clathrin facilitating uptake of QDs

which partially explains why there was higher uptake into female cells. This finding provides empirical evidence into how the physicochemical characteristics of the protein corona can affect NP-cell behavior; here we see how the protein corona profile can affect uptake of quantum dots [34].

The cytoskeletons were then analyzed to examine structural differences to further explain differences in quantum dot uptake. Via the use of stochastic optical reconstruction microscopy, actin filaments were found to be organized differentially between sex-specific hAMSCs implying that there could be differences in uptake and trafficking of QDs. The results indicate that filaments are arranged differentially with respect to bundles, density, and direction. After treatment with quantum dots, it was found that quantum dots localized to actin filaments, structures which help to facilitate endocytosis. Sex differences were then tested in the uptake of quantum dots in clathrin-coated vesicles, proteins that coat vesicles that are endocytosed via the plasma membrane to aid in endocytosis. From this experiment, the results suggested male hAMSCs accumulated more clathrin than female hAMSCs. To test whether cell type affects QD uptake in sex-specific cells, the experiments were repeated in primary fibroblasts from a male and female patient of similar age which showed that male fibroblasts had greater uptake instead concluding that sex-based differences were present. The differences in QD uptake and clathrin-coated vesicles in different cell types largely boiled down to the fact that the male fibroblasts had a greater amount of actin filaments allowing for increased uptake into cells [34].

However, this did not hold true when the fibroblasts were treated with gold NPs (AuNPs). The results suggest that AuNP uptake is non-specific meaning that uptake can be mediated by endocytosis or direct penetration through the plasma membrane. Unlike the QDs, the NPs were not localized to the actin filaments. Instead, they were more diffusive throughout the cytosol [34].

This work highlights many factors that can affect nanotechnological efficacy as a result of sex including protein secretion, cytoskeletal differences, and physicochemical characteristics. This work could be improved by incorporating more variables known to affect health outcomes including geographical/genetic ancestry and age. For instance, the cytoskeleton can undergo remodeling during natural aging which can affect the cell cycle, polarity, and cell migration [43]. This work could also benefit from including size data for the QD experiments to support sex-differences in uptake claims. The size of the NPs/QDs are known to affect protein corona recruitment which, in turn, affects uptake into cells [44].

Considering biological sex in future nanomedicines

The field of sex-based nanomedicine in humans is in its infancy; there has not been nearly enough progress in this field. More in vitro and ex vivo experiments need to be conducted to test sex as a biological variable. Echoing a previous statement, harvesting samples from a specific organ could provide insight into what proteins and biomolecules promote accumulation, immune response, and cytotoxicity. Future studies testing biological sex as a variable should also consider de-coupling sex and gender which can be done by testing embryonic cells for sex differences. An interesting way that confounding variables have been controlled for is by generating isogenic induced pluripotent stem cells from a Klinefelter syndrome patient. iPSCs containing XX and XY cells were made from the XXY parent cells to isogenically study sex differences [45]. Theoretically, these cells should contain identical genetic makeup from ancestry to disease state other than biological sex.

The effects of genetic ancestry on protein corona

Difference between race and ancestry

Race and ancestry are often convoluted much like sex and gender. However, it is important to delineate between the two since both are known to affect health outcomes in distinct ways. Race and ethnicity are social constructs based on societal perception and biases and were a means to justify slavery, segregation, and socioeconomic inequities [46, 47]. Notably, race and ethnicity have been shown to have no biological basis [48]. However, it is irrefutable that structural and institutional racism continues to persist as a factor in determinants of health [48]. For example, Black and Hispanic populations experienced significantly higher prevalence, hospitalization, and mortality rates due to COVID-19 than White people underscoring the need to address and improve health inequity [49].

Ancestry refers to one's family origin and heritage [46, 47]. Furthermore, ancestry focuses on one's biological/genetic background and geographic origin rather than skin color in order to identify relevant markers that show significant differences in frequencies between populations [47]. Therefore, ancestry is a more inclusive and clinically relevant factor that can be used to improve accuracy and efficacy of medicines. Research suggests variance in drug metabolism between different ancestral groups. For example, dosing for eltrombopag olamine was reduced by 50% for patients of East Asian descent due to a 15% incidence of hepatobiliary plasma abnormalities compared to 5% incidence in Caucasians [50]. However, it was undeterminable if genetic predisposition was the cause of this disparity. Investigation

into ancestral/genetic determinants of health could help improve efficacy of medicines.

Nanotechnology, ancestry, and the protein corona

To our knowledge, there is no research investigating ancestry as a factor in the performance of nanotechnologies nor the protein corona. Therefore, here, we describe variances in proteome analysis between different ancestral backgrounds which could influence the protein corona.

Sjaarda et al. evaluated the impact of ancestry on an admixture of 237 serum biomarker concentrations in diabetes patients via the use of variance component modeling [51]. Results indicated that ancestry affects 19% of biomarkers with 5% having significant phenotypic variance by more than 10%. The region at rs4149261 was associated with increased levels of C-peptide, and increased risk of diabetes and insulin resistance. This was found to be an effect of African ancestry; however, it was not specified what region of Africa was tested. Further hypothesis testing found that 5% of the biomarkers were affected by ancestry with 30% showing significant differences. The findings from the researchers' models suggest that "biomarkers harbor true biological inter-ancestry differences in contraction that are genetically determined" [51]. With these differences in biomarker concentrations, one can expect differential protein corona absorption when administering a nanotechnologically-based therapeutic for diabetes treatment due to variances in C-peptide concentration and other biomarkers. This, in turn, can affect the performance and uptake of the therapeutic due to physicochemical modifications to the surface of the NP including size, zeta potential, etc.

Many studies have investigated protein quantitative trait loci (pQTL) to look at genetic regulation of proteome circulation and how it relates to disease susceptibility and predisposition. Xu et al. identified pQTL to identify any genetic control in proteome circulation in Han Chinese in addition to providing insights into disease susceptibility between groups of East Asian descent and European descent [52]. Results indicated that 45 out of 60 protein-phenotypes were not previously identified in Europeans. Out of the 41 pQTLs in both European and East Asian populations, genetically determined body mass index (BMI) was positively associated with 28 proteins and negatively associated with 2 proteins in European. Analysis in the East Asian population indicated 34 non-significant negative associations with BMI implying that the two populations may have varying genetic predisposition to obesity. Different proteins were also identified between East Asian and European populations that may suppress BMI effects on type II diabetes and coronary artery disease. Haptoglobin, alpha-1-antitrypsin, heparin factor 2, factor H, and C4b-binding protein alpha chain were suggested to suppress the effect of BMI on type II diabetes in European populations. Haptoglobin, heparin factor 2, factor H, inter-alpha-trypsin inhibitor heavy chain H3, and kininogen-1 were also suggested to suppress BMI effects on coronary artery disease in European populations. These proteins were not observed to have the same effect in the East Asian population. This cross-ancestry analysis revealed ancestral-based differences in proteomics which could point to disease susceptibility and prevalence between populations with different geographical origin [52]. In nanotechnology, disease-specific protein corona is known to form on the surface of NPs which can affect the uptake, performance, and efficacy of the therapeutic. Further discussion of disease state and the protein corona can be found in "The effects of disease state on protein corona" section.

Kachuri et al. took a similar approach in looking at pQTLs in African Americans, Puerto Ricans, and Mexican Americans [53]. They revealed greater genetic variance in whole-blood gene expression in African Americans followed by Europeans and Indigenous Americans. Additionally, they also found that 30% of heritable protein-coding genes are ancestry specific. These genes are very rare or do not exist in other ethnic populations, and this finding stays consistent when the defined ancestral boundaries were relaxed.

Zhang et al. developed models for plasma protein imputation for European and African populations to pinpoint drug targets for gout [54]. Their analysis revealed that 30% of the sentinel pQTL in the African populations was nonexistent or extremely rare in European populations. Conversely, 10% of the sentinel pQTL in the European populations was nonexistent or extremely rare. When applying these models cross-ancestry, it was found that applying African models on to the European populations performed better than the converse [54]. Again, this highlights the effect that geographical ancestry can have on plasma proteome composition due to the fact that the protein imputation models perform differently across ancestry. NPs that are designed to target a specific protein may not develop the appropriate protein corona in order to have their intended effects on different ancestral groups since proteomes may not be common. Hence, this work also highlights the need to develop specific models for different ancestral groups to further develop formulations for nanotechnologies.

Zhou et al. determined that increased levels of 2′-5′-Oligoadenylate Synthetase 1 (OAS1), an antiviral protein that facilitates innate immune responses against RNA viruses, is strongly associated with decreased rates of very severe COVID-19, hospitalization, and susceptibility in European populations [55]. Populations outside

of Sub-Saharan Africa are known to harbor protective alleles rs4767027-T (OAS1 pQTL) and rs10774671-G (OAS1 sQTL) which differ due to evolutionary history; the rs4767027-T allele was derived from Neanderthal lineage whereas rs10774671-G allele is preserved from Neanderthal lineage. Additionally, the rs10774671-G allele regulates alternative splicing which increases levels of the OAS1 p46 isoform and has greater activity against the p42 isoform. The findings indicate that p46 isoform levels and protective effects on COVID-19 outcomes are positively associated; however, this finding is exclusive to the European population. Data is lacking on if the p46 ancestral allele preserved in Sub-Saharan Africans also offers protection. Here, it was shown that differences in plasma proteome has a genetic basis stemming from Neanderthal ancestry which offers benefit in COVID-19 risk modulation further emphasizing ancestral-based differences in proteome composition. The OAS1 forms prevalent in proteomes of different ancestral groups may offer variable immune responses when treated with nanotechnologically-based therapeutic. The increased potency of p46 against p42 could provide a genetic and biological basis into differences in disease susceptibility as well as drug metabolism for viral illnesses [55].

Considering genetic ancestry in future nanomedicines

The field of nanomedicine could benefit from more research regarding the heritability of the proteome; there is very limited research investigating groups of similar geographical origin. Further research could include in vivo studies looking at heritability of proteome in mice. In addition, mice could be treated with NPs from generation to generation whilst observing changes in the protein corona and immune responses. Ex vivo studies investigating the protein corona around NPs could also be conducted with primary human plasma of similar geographical origin to continue to identify common genes linked to the proteome. This could also help diversify the proteome profile for ancestral groups and help refine models such as those used by Zhang et al. Lastly, a vast amount of these studies fail to consider that populations from different regions in Africa can vary widely in genetic ancestry, but are commonly grouped together in studies.

The effects of biological age on protein corona

Difference between chronological age and biological age

Chronological age and biological age represent intertwined yet distinguishable concepts. It is crucial to unravel their intricacies, as both chronological and biological age exert unique influences on health outcomes, shaping our understanding of aging and its associated determinants. Chronological age, as defined by the amount of time elapsed since birth, serves as a fundamental determinant of health outcomes, chronic diseases, and mortality [56]. Biological age on the other hand, represents an individual's physiological state and is influenced by various factors, including genetics [57, 58], lifestyle [59, 60], and environmental stressors [61, 62], which in turn can affect normal biological functions such as cell renewal, senescence, and cell death, as well as modify the DNA [63]. In simpler terms, biological age is a determinant of our tissues, cells, and organ systems' age and is seen as a better life expectancy measure compared to chronological age [64]. The easiest way to understand the difference between the two is by looking at the substantial heterogeneity in the health trajectories of older individuals. For example, an elderly individual might require assistance to do daily tasks, while someone of the same chronological age might not until later years [65].

Weathering and biological age

With that in mind, the biological age also encapsulates the accelerated aging resulting from cumulative weathering caused by exposure to social-economic [66, 67] and political discrimination [68], psychological stressors on individuals as well as healthcare disparities [69], particularly in racial minority populations. For example, according to a study conducted by Forrester et al. [70], weathering was investigated by comparing the chronological and biological age of middle-aged non-Hispanic African American and Caucasian individuals. As a result, the mean biological age for African Americans was 57.1 years which was 2.6 years older than their chronological age, while the mean biological age for Caucasian was 52.3 years which was 3.5 years younger than their chronological age, confirming racial differences in weathering.

Another interesting study conducted by Simons et al. [71] examined the effect of economic differences in terms of income and financial pressure on biological weathering in a random population of 100 middle-aged Black women. Financial pressure encompasses psychological stressors caused due to difficulty paying rent, bills, and or daily necessities such as food. The study uses an epigenetic measure of biological aging based on methylation changes at 71 CpG sites according to Hannum et al. The main parameter that was investigated in this study was household income ranging from $10,000 to $200,000. Other socioeconomic factors such as education, marital status, and childhood trauma were controlled to focus on only the contribution of economic factors to accelerated aging. Intriguingly, the findings revealed a strong correlation between low income and accelerated aging, with approximately 68% of women with per capita incomes below $3900 exhibiting accelerated aging. On the other hand, 70% of those with per capita incomes above

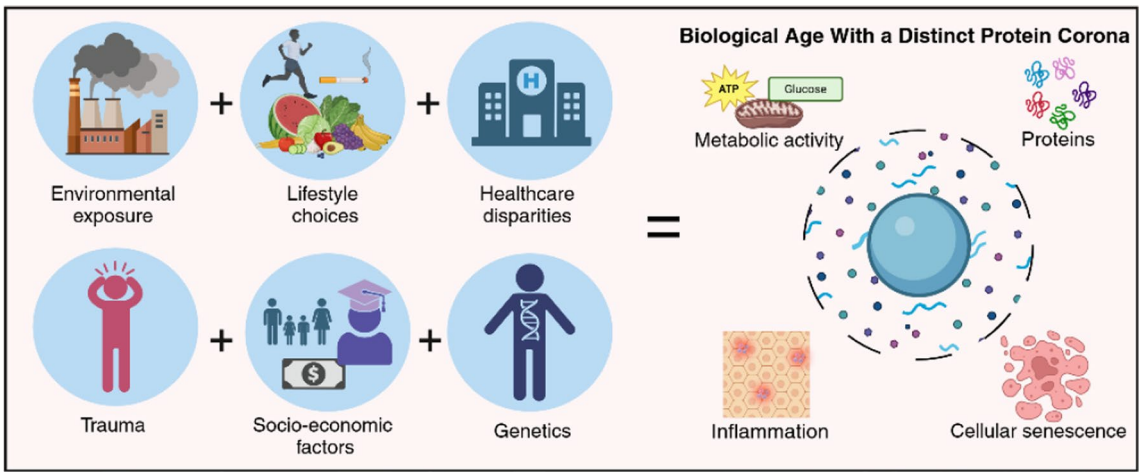


Fig. 4 Factors influencing biological age and their impact on protein corona formation. Created with BioRender.com

$15,000 experience decelerated aging. Low income can have an impact on social life and health-related behaviors such as limited access to exercise, and or dietary choices which in turn might cause health problems characterized by a high body mass index (BMI). It might also promote participating in activities for stress relief such as consuming alcohol and smoking which is detrimental to health. Thus, the study further investigates various health-related behaviors, including diet, exercise, smoking, alcohol consumption, and access to health insurance. Surprisingly, the results suggested that these behaviors did not have a significant influence on accelerated aging.

Researchers have also assessed the effects of post-traumatic stress disorder (PTSD) and trauma on accelerated aging, as measured by plasma *N*-glycosylation [72]. *N*-glycosylation refers to the attachment of a sugar chain, referred to as a glycan, to the nitrogen atom of a protein or other molecule. Glycosylation modifications often affect plasma protein structure and function [73]. The study by Moreno-Villanueva et al. involved the GlycoAge Test—a log ratio of *N*-glycan measurements—which is a known biomarker for biological aging [72]. Blood was collected from 32 participants classified in either 'PTSD', 'high-stress or trauma-exposed', or 'low-stress' groups. Key findings revealed that individuals with PTSD and trauma showed increased values in the GlycoAge test equivalent to approximately 15 years of additional aging, with a positive correlation between measured traumatic load and the GlycoAge Test. These findings suggest that high stress affects *N*-glycosylation patterns, thus affecting plasma protein behavior and biological aging processes. These results corroborate knowledge that stress and trauma contribute to premature aging and reveal further insights into the molecular mechanisms of biological weathering.

Understanding the distinction between chronological age and biological age is crucial in comprehending the nuanced effects of aging on biological systems, particularly in the context of NP protein corona formation. In our review, we are specifically using biological age as the scientific variable due to its comprehensive reflection of an individual's physiological state and its susceptibility to external stressors such as trauma and socio-economic factors. Trauma, both physical and psychological, influences aging processes at a molecular level, yet has been overlooked in most aging research. Recent studies using epigenetics reveal trauma's potential to accelerate aging through metabolic changes akin to those seen in aging [74, 75]. Inflammation, a common feature in both trauma response and aging, has been increasingly recognized as a driving force behind age-related ailments. The chronic inflammatory state, often termed 'inflammaging' [76], characterized by elevated pro-inflammatory cytokines and reduced anti-inflammatory cytokines, has been associated with increased mortality, frailty, and age-related diseases. Trauma-induced inflammation, oxidative stress, and DNA damage may accelerate aging by shortening telomeres, vital markers of cellular aging. Additionally, trauma may increase cellular senescence, potentially exacerbating aging-related ailments [76]. The cumulative effect of trauma-related stress can thus significantly alter biological age, making it a critical factor in aging research. Thus, biological age, as opposed to chronological age, offers a more precise measurement of how aging impacts biological processes. This precision is essential when investigating the interaction between aging and protein corona formation. By focusing on biological age, we aim to explain the distinct influences of aging on protein corona formation, providing deeper

insights into the relationship between aging, biological responses, and the interaction with NPs.

Beyond the specific metric of biological age, we are also investigating the broader concept of how we age and its impact on protein corona formation. Aging is a multifaceted process influenced by various factors, as illustrated in Fig. 4. These include genetic predispositions, environmental exposures, lifestyle choices, and cumulative stressors over a lifetime. These factors collectively shape the aging process and can lead to diverse health outcomes. By studying how aging affects the formation and composition of the protein corona, we aim to understand how age-related changes in the body's biochemical environment influence the behavior of NPs. This research is critical because the protein corona, which forms around NPs when they enter a biological system, plays a crucial role in determining the biological identity and fate of the NPs. Variations in protein corona composition due to aging could impact the efficacy and safety of NP-based therapies [77]. Therefore, our investigation will focus on identifying age-related changes in protein corona formation, with the goal of optimizing NP design for improved therapeutic outcomes in older populations.

Nanotechnology and biological age

Biological age, which encompasses the functional and physiological state of an individual rather than just chronological age, can significantly influence the performance and efficacy of nanotechnology in medicine. Aging affects the immune system, leading to a condition known as immunosenescence [78, 79], which is characterized by a decline in immune function and reduced responsiveness to vaccines and therapies. A study by Connors et al. [78] demonstrated that empty lipid NPs (eLNPs), a component of mRNA-based vaccines, induced different immune responses in young and aged individuals. Specifically, eLNPs promoted maturation and cytokine production in dendritic cells and monocytes, but these effects were diminished in older adults, who exhibited decreased CD40 expression and interferon production. This suggests that the aging immune system may not respond as effectively to NP-based treatments, impacting their therapeutic efficacy.

Understanding these age-related differences is crucial for tailoring nanomedicine approaches. For example, aged individuals showed dysregulated antiviral signaling and reduced phagocytic activity, highlighting the need for age-specific formulations to enhance immune responses [78]. Additionally, the study revealed that aged individuals had impaired responses to SARS-CoV-2 vaccines, with less robust and shorter-lived immune responses compared to younger individuals. This highlights the importance of considering biological age when designing NP-based therapies, to ensure they are effective across different age groups. By incorporating these insights into NP design, researchers can develop more effective nanomedicine strategies that account for the physiological changes associated with aging, ultimately improving treatment outcomes for older populations.

Biological age and the protein corona

The impact of biological age on protein corona formation is multifaceted, however often overlooked in nanotechnology research. To understand the biological aging process, organismal and cellular aging also known as cellular senescence stands as an accurate representation [80]. Cellular senescence is a state in which a cell undergoes a permanent and irreversible growth arrest and resistance to dividing and proliferating [81]. Like biological aging, cellular senescence is also triggered by various epigenetic and environmental stressors and causes subcellular damage such as damage to the mitochondria and the DNA, thus becoming the precondition for anatomical aging [81, 82].

Cellular senescence has indeed been shown to affect NP performance. In a study conducted by Foroozandeh et al. [83], the effect of cell aging induced through replicative senescence and exposure to oxidative and genotoxic stress on the toxicity of specifically the PEGylated QDs was investigated. The study involves using the healthy untransformed IMR90 fibroblast cells from a 16 week-old Caucasian female human fetal lung and CCD841CoN epithelial cells from a 21-week-old (unknown ancestry) female human fetal colon at passage number below 10. In both cell types, senescence was induced and compared to young cells. The results reveal that the preconditioned medium from young cells caused QDs to be less stable and more prone to agglomeration compared to the medium from senescent cells. This instability in young cells was thought to be due to the higher secretion of extracellular proteins which can alter the protein corona composition, however, this was not analyzed or confirmed in the study. Senescent cells also exhibited increased susceptibility to QD toxicity compared to young cells in both cell types. NPs were soaked in fetal bovine serum to develop a hard corona and it was concluded that the presence of this hard protein corona can mitigate observed NP toxicity by delaying QD disintegration and reducing immediate cytotoxic effects. The findings also revealed that there was a delay in cell death induction for protein corona-coated QDs. This delayed induction of cell death in the presence of a protein corona implies that the corona may play a protective role by reducing the immediate cytotoxic effects of QDs. Overall, the findings emphasize the importance of considering cell age as a factor for tailoring nanomaterials to be used

in clinical applications for different age groups. However, this work used only fetal bovine serum to develop hard NP coronas and future work should seek to characterize the effects of age on protein corona composition.

It has been shown in the literature that senescence can induce differences in protein secretions. In a study conducted by Philip et al. [84], key age-associated phenotypic changes in human cells were investigated by integrated biophysical and biomolecular properties to improve the understanding of cellular aging. The study involved the use of primary human dermal fibroblasts obtained from donors aged 2 to 96 years for a cohort of 32 samples. Among the biomolecular characteristics, the study detailed protein secretion levels using high-throughput single-cell secretion microchip technology which profiled the secretion of 23 different proteins. Among these, IL-6 was identified as a key proinflammatory cytokine with a significant correlation to age ($\rho = 0.52$) and a significant increase in secretion with age. This suggests that as cells become senescent, they secrete higher levels of IL-6, which could influence the composition of the protein corona on NPs, potentially affecting their stability and interactions with cells as well.

In another study conducted by Waldera Lupa et al. [85], proteins secreted by senescent normal human dermal fibroblasts (NHDF), and their potential impact on the extracellular matrix (ECM) and inflammatory processes were investigated. The study involved culturing NHDFs and collecting their secreted proteins for analysis. Mass spectrometry was used for protein identification and validation, along with bioinformatics tools to determine the secretion signals and classify the proteins as secreted. Key findings from the study revealed that senescent cells secrete a variety of proteins, including interleukins such as IL-1B, migration inhibitory factor, SERPINB/PAI (plasminogen activator inhibitor) 2, and FGF2. Additionally, membrane-associated proteins like CD14, Cadherin-2 (CDH2), and tissue factor pathway inhibitors (TFPI) were identified. The study also noted age-associated increases in cytokines such as IL-1B, IL-1RN, IL-4, IL-15, interferon-gamma (IFNγ), C-X-C motif chemokine ligand 10 (CXCL-10), and tumor necrosis factor-alpha (TNFα). Collectively, these studies suggest that the secreted proteins from senescent cells could significantly alter the composition of the protein corona around NPs, potentially affecting their biological interactions and therapeutic efficacy.

In line with the work showing that senescent cells produce different proteins, protein composition in serum has been shown as a marker of biological aging. Multiple studies suggest that circulating plasma proteins change with biological age and contribute to accelerated aging and health decline [86]. The growing field of proteomics has particular importance in nanotechnology due to the significant influence of plasma protein composition on protein corona and NP behavior [87]. For instance, researchers investigated the impact of age on plasma proteins, which are known to affect protein corona composition [88]. The study involved 4263 human plasma samples from donors aged 18–95 years. Researchers quantified 2925 unique proteins using advanced proteomic techniques, and then processed them with bioinformatic models. Age-associated changes in the proteome were correlated with known biological aging markers. Results revealed that 373 of measured plasma proteins highly accurately predict biological age ($\rho = 0.97$). Proteins that changed most dramatically with age included SOST, ARFIP2, and GDF15. Researchers found that accelerated and decelerated biological aging relies on aging pathways, with 3 significant waves of changes in plasma proteins at 34, 60, and 78 years of age. These waves were also accompanied by physical and cognitive function decline, indicating that these protein changes were physiologically significant.

Interestingly, another study by Lehailler et al. indicated that immune proteins were particularly valid in predicting biological age, and many immune proteins revealed increased expression with biological age, triggering heightened inflammation [89]. This group created a predictive 'aging clock' based on 491 proteins with a median prediction error of 2.44 years. These variations in protein concentrations based on age may indeed affect protein corona formation. In a study conducted by Kuschnerus et al. [90], researchers explored the strategy of inducing a fibrinogen protein corona onto gold NPs to alter their behavior. While fibrinogen is not explicitly considered a biomarker for biological age, its association with cardiovascular, cerebrovascular, and other age-associated diseases is well-documented, and one study recently correlated fibrinogen levels with predicted biological age based off glycosylation levels [91–93]. Thus, higher levels of fibrinogen can be said to be associated with biological age. Kuschnerus et al. incubated gold NPs in fibrinogen and bovine serum and performed dynamic light scattering and gunshot proteomics to characterize the protein corona. The results revealed that NPs in the presence of fibrinogen exhibited higher agglomeration, fibrous structures, and intracellular oxidative stress. Uptake by microglia was also higher with fibrinogen protein coronas. These findings imply that the presence of age-associated plasma proteins in protein corona, including fibrinogen, can drastically affect NP properties and behavior. As levels of these proteins vary with biological age, it is important to consider proteomic age as a factor when designing nanomaterials for diverse populations.

Considering biological age in future nanomedicines

Biological aging represents a crucial physiological process that shapes bodily interactions with nanotherapeutics. With recent advances in epigenetic clocks and machine learning models, it has become more feasible to assess biological age, offering a more personalized assessment of health risks compared to chronological age. This personalized approach can address the physiological effects of health disparities that may affect nanomedicine efficacy at the molecular level. Cellular senescence and the plasma proteome represent important components of biological aging that influence the protein corona of NPs. Further research must be done to evaluate the effects of accelerated aging on the protein corona, and the resulting NP behavior. Potential new avenues of research include examining how biological age affects protein corona composition, NP uptake, and cellular viability, as well as designing NPs tailored to diverse proteomic and senescent secretion profiles. Understanding aging-related processes in the context of protein corona formation may lead to innovative advances in the development of personalized NP-based therapies for diverse patient populations.

The effects of environment on protein corona

Difference between biological and external environment

It is essential to distinguish between one's biological and external environments. In the context of nanomedicine, one's biological environment describes the surrounding proteins, carbohydrates, lipids, and other substances that could interact with a therapeutic once inside a patient. External environment refers to physical elements that impact one's biological environment, such as air pollution.

External environment is a well-documented contributing factor to healthcare outcomes. Air and water quality, diet, and weather patterns are all known to impact different aspects of human health. For example, carbon monoxide, one of the most common air pollutants, is associated with altered neurodevelopmental outcomes in children [94]. Excess exposure to heavy metals (such as lead) via contaminated water is also linked to neurological and cardiovascular dysfunction [95]. Further, weather patterns are correlated with changes in mental health states. Millions of people suffer from seasonal affective disorder, which describes depressive episodes that typically begin during the winter months and resolve in the summer [96]. Seasonal affective disorder is often successfully treated with light exposure, indicating a relationship between overcast skies (which are commonplace in winter) and worsened mental health. Doubtless, one's external environment alters health and likely impacts protein corona composition. The primary focus of this section is said impact.

External environment and protein corona

Effects of diet on the protein corona Diets depend on what foods are readily available in one's immediate surroundings. If someone lives in a coastal region, they are more likely to eat seafood than someone in a landlocked environment [97]. The prevalence of food deserts can cause further diet differentiation within a given area, with some residents having access to fresh local fare and others forced to turn towards highly processed foods. Due to this tight tie-in with location, diet is an external environmental factor for the purposes of this work.

Recent research has emphasized the importance of an enzyme corona surrounding orally ingested NPs. Digestive enzymes are thought to rapidly adhere to the surface of NPs once inside the gastrointestinal tract [98]. Once attached, these enzymes can affect numerous aspects of NPs that determine their functionality in vivo, such as size, morphology, and toxicity to surrounding tissues [99]. These significant changes can impact the functionality of NPs. Furthermore, specific digestive enzymes associate with different NP types (Table 4). Foods that alter the production and function of digestive enzymes, such as tea phenols, may impact orally administered NP's biological fate and protein corona composition [100].

Recent work has also found many interactions between milk proteins and food-grade SiO2 and TiO2 NPs. Particles were suspended in a solution of skimmed milk for 1 h at 37 °C. Particles were removed from the solution via centrifugation and subsequently subjected to SDS-PAGE and MS to determine the composition of their protein coronas. For silica NPs (SiO2), proteins such as β-casein exhibited higher adsorption due to the particles' larger hydrodynamic size (~ 583.78 nm) and highly negative zeta potential (− 26.25 mV). These factors, along with high hydrophobicity, facilitated greater protein binding. Protein characteristics, including beta strands and specific amino acids (Ile, Tyr, Ala, Gly, Pro, Asp, Arg), also influenced adsorption. For titanium dioxide NPs, adsorption was notably influenced by the presence of hydrophobic amino acids and the protein's isoelectric point. Phue and their team also examined the most commonly bound proteins on each particle type, finding that SiO2 NPs had an apparent affinity for complement factor H, C4b-binding protein alpha chain, fibronectin, and fibrinogen beta chains, whereas TiO2 NPs often interacted with fatty acid-binding protein, heart, and sodium-dependent phosphate transport protein 2B [101].

Additionally, research highlighted by Ke et al. described whey proteins' ability to confer "stealth" to NPs [102]. In this context, stealth refers to NPs' ability to bypass

Table 4 Digestive Enzyme Interaction with Various NPs (Data attributed to Zhang et al. [98])

NPs	Size/zeta potential	Associated digestive enzyme	Enzyme-NP interactions	References
Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) cationic NPs	268.6 nm; +26.5 mV	Pepsin and pancreatin	Size: Increased to over 340 nm in SGF with pepsin and to over 400 nm in SIF with pancreatin; Zeta Potential: Increased to + 33 mV in SGF with pepsin and converted to a negative value in SIF with pancreatin; Morphology: Enzyme corona formed; Uptake: clearly reduced in Caco-2 model	Peng et al. [141]
Polystyrene NPs	~ 100 nm	Pepsin, a-amylase and Trypsin	Size: pepsin-NPs (200 nm), a-amylase NPs (150 nm); Zeta Potential: In SGF with pepsin, − 10 to − 3 mV, in SIF with amylase, − 27 to − 18 mV, in SIF with trypsin, − 27 to − 17 mV; Morphology: Corona Formed	Wang et al. [99]
Lactoferrin (LF)-encapsulating carboxymethyl-chitosan NPs	[LF] = 0.2 mg/ml: 126.6 nm; + 3.6 mV [LF] = 0.5 mg/ml: 654.6 nm; − 2.3 mV	Pepsin and trypsin	Both formulations showed resistance to pepsin & trypsin digestion; Showed antimicrobial properties at ~ 400 ug/ml	Hedyeloo et al. [142]
Poly(maleic)-alt-1-octadecene coated magnetite (Fe_3O_4) NPs	~ 50 nm; − 21 mV	Pepsin, lipase, amylase, trypsin and chymotrypsin	Size: Two times larger in SSF, three times larger; Morphology: corona formed; uptake: enhanced in Caco-2 cell model	Silvio et al. [143]
ZnO NPs	1976 nm in distilled water; + 16 mV	Pepsin and pancreatin	Size: Increased to over 2300 nm after incubation for 1 h; Zeta Potential: converted to negative values; Bioavailability: no significant change	Yu et al. [144]
Edible Dock Protein (EDP) NPs (loaded with Quercetin)	Original Zeta Potential: − 17.9 mV to + 3.8 mV	α-amylase, Pepsin, Trypsin, Amylopsin	α-amylase: Increased zeta potential from − 17.9 to − 11.2 mV; Pepsin: Increased zeta potential from + 3.8 to + 7.0 mV; Trypsin: Increased zeta potential from − 21.9 to − 19.2 mV; Amylopsin: Increased zeta potential from − 21.9 to − 12.1 mV. Size, PDI, and protein conformation significantly changed after corona formation. Activity of α-amylase and amylopsin was enhanced by 1.4 and 1.6 times, respectively. α-amylase and amylopsin coronas slowed the release of quercetin, while pepsin and trypsin coronas accelerated its release	Wu et al. [145]
NH_2-Starch NPs	120 ± 2.6 nm; + 23.2 ± 1.0 mV	Pepsin	Size: Increased to 203 ± 12.2 nm in SGF; Zeta Potential: reduced to 12.7 ± 0.2 mV in SGF. Pepsin-protein corona binding induced secondary structure change in pepsin without significantly decreasing efficacy	Wang et al. [146]

Table 4 (continued)

NPs	Size/zeta potential	Associated digestive enzyme	Enzyme-NP interactions	References
Ag NPs	41.4 ± 3.8 nm	Pepsin	Formation of a hard enzyme corona with pepsin led to agglomeration and flocculation. Pepsin protected AgNPs from dissolution in acidic SGF	Jeong et al. [147]
Ag NPs	57 nm	Pepsin and Pancreatin	Size: Increased to 570 nm in pepsin-containing SGF; Morphology: surrounded by enzymes and agglomerated	Pinďáková et al. [148]
TiO_2 NPs	163.9 ± 3.3 nm in water; 208.7 ± 18.0 nm after SGF incubation	Pepsin	Formation of soft protein corona with pepsin; size increased by ~ 27% in SGF. Zeta potential shifted from positive in water to slightly negative in SGF due to weak electrostatic interactions. Pepsin activity reduced by 8.5%, fluorescence quenching observed, but no significant change in secondary structure	Sun et al. [149]

AgNP silver NP, *GIT* gastrointestinal tract, *NP* NP, *SGF* simulated gastric fluid, *SIF* simulated intestinal fluid

detection and destruction by the immune system [103]. Whey proteins (caseins, various chaperone proteins, β-lactoglobulin) and lecithin can coat NPs, preventing other proteins from binding and inhibiting elimination from the bloodstream. These findings demonstrate the direct impact one's diet can have on protein coronas and the need to characterize interactions between NPs and common foods.

Effects of pollution and climate on the protein corona There is limited research discussing environmental pollution and its effects on protein corona formation. However, certain pollutants, such as particulate matter less than 2.5 um in diameter and metal-containing NPs like Fe3O4, can denature proteins [104]. These denatured proteins adsorb to NPs similarly to their properly folded counterparts, which can trigger unfolded-protein response pathways in vivo. Liu et al. demonstrated that inhalation of these pollutants caused massive inflammatory cell infiltration in the respiratory system in a murine model, likely due to raised levels of denatured proteins and unfolded-protein response pathway expression.

Furthermore, heavy metal exposure can alter the behavior of NPs in vitro. Frost et al. examined the impacts that commonplace heavy metal ions such as cadmium (Cd), lead (Pb), mercury (Hg), and nickel (Ni) had on gold-citrate and silver nanoparticles. Notably, Pb ions caused rapid aggregation of AuNPs but not AgNPs, demonstrating that the type of NP material influences metal-NP interactions. Pb exposure in an acidic environment also caused a substantial reduction in the surface charge of AuNPs, which led to particle destabilization and aggregation, a behavior not duplicated with other ions [105]. Additionally, ionic-gelation-prepared chitosan NPs demonstrated a high sorption capacity for Pb ions (maximum adsorption of 398 mg/g). Using atomic force microscopy, Qi and Xu found that the nanoparticles, although initially dispersed in solution, became surrounded by lead ions, leading to NP aggregation [106]. Once accumulated in the body, heavy metals can trigger the production of reactive oxygen species, which are highly cytotoxic and can modulate gene expression, consequently altering an organism's proteome [107, 108]. These findings display heavy metal ions' ability to directly influence nanoparticle interactions and proteomic profiles, thus impacting protein corona formation and composition.

Considering the global prevalence of heavy metal pollution in water supplies and these pollutants' potential to disrupt NP stability and biological systems poses a significant concern for both environmental and human health [109]. Going forward, researchers should examine the interactions between commonplace pollutants (heavy metals, particulate matter, plastics, etc.) and different proteins in the human body. Should a toxic pollutant bind to a specific protein, and that protein, in turn, adsorb to NPs, then this complex could easily pose a danger to cells throughout the body, potentially causing increased cytotoxicity or disrupting metabolic processes. Furthermore, NP-pollutant binding could also render delivery systems ineffective via increased aggregation, as seen in AuNPs.

Once again, there is minimal research on climate and its resulting impact on protein corona composition. It is well-documented that exposure to ultraviolet (UV) radiation can affect gene expression [110]. Key genes affected by UV exposure include SLC45A2 (which encodes for a membrane-associated transporter protein that impacts skin color), KRT77, and OCA2, which are also involved in skin pigmentation and have shown adaptation in response to varying levels of solar radiation [111]. UV exposure also upregulates the expression of genes responsive to interferon-gamma in melanocytes, leading to inflammation and potentially contributing to melanoma development [112]. Proteomic differences based on climate are also poorly characterized. Known climate-based proteomic variance is limited to skin pigmentation proteins, those involved in synthesizing vitamin D, and folate catabolism [113].

Considering environment in future nanomedicines

The potential impact of one's external environment on protein corona composition and drug delivery at large remains an understudied area. Future research should examine how various commonplace diets (American, Mediterranean, Paleo, Keto, etc.) impact serum levels of different proteins and how those proteins interact with a wide array of NP types. Furthermore, in the last two decades, plastics such as bisphenol-A have been shown to alter methylation patterns early in development, thus changing the proteomes of those exposed in utero [114]. This could foreseeably impact protein corona formation, considering its effects on long-term proteome composition. Other pollutants, such as heavy metals and other plastics, should be examined for their ability to alter gene expression at various stages of development. Researchers could identify a population that is overexposed to a given pollutant and collect data on their experience with different classes of drugs. Bloodwork and survey data regarding patient experience could be collected to see whether the population had an unusual experience with a specific type of drug. If such a correlation is observed, further work could consist of characterizing that pollutant's effects on gene expression and protein corona composition. A similar study could be conducted to investigate climate and its impact on drug metabolism. To aid in this effort, further research is needed to understand better

differential gene expression based on climate and how this alters protein corona composition.

The effects of disease state on protein corona

Diseases and the protein corona

All types of diseases (infectious, hereditary, deficiency, and physiologic) can alter gene expression and an organism's proteome [115–118]. For example, infectious diseases commonly induce the production of cytokines, antibodies, acute phase, and complement proteins [119, 120]. Additionally, hereditary diseases are exclusively caused by inherited genetic mutations, which can lead to down or upregulated expression of one or more proteins [121]. Any change in serum levels of proteins in the body, such as those described above, can change protein corona composition. Even among individuals with the same disease, protein coronas can differ significantly, reflecting the highly personalized nature of these structures [122, 123]. This variability in protein corona composition could be instrumental in designing NP-based diagnostic systems tailored to bind rare proteins, miRNAs, peptides, metabolites, and specific cell types. To this end, NPs could be engineered to undergo conformational changes, fluoresce, or bind to a wide array of proteins. Additionally, understanding which proteins bind to specific nanoparticles, and in what quantities, in particular disease states can provide valuable insights for improving targeting and diagnostic strategies in NP-based delivery systems.

Cancer and the protein corona Recent work has identified numerous cancer-specific alterations to protein corona composition. Such changes have been exploited for diagnostic purposes, as seen in work undertaken by Zheng et al., who developed a two-step assay for early prostate cancer detection [124]. Citrate-capped AuNPs were first incubated in sera from patients with malignant (n = 32) and benign (n = 23) prostate tumors and were subsequently subjected to DLS and LTQ-Orbitrap mass spectrometry for size and protein corona characterization, respectively. Notably, AuNPs exposed to cancer-positive sera displayed increased adsorption of IgG polypeptides (heavy, kappa & lambda chains specifically). The authors attributed this phenomenon to adaptive immune responses against tumor-specific antigens circulating in the patients' bloodstreams. These responses, characterized by the production of anti-tumor autoantibodies, likely caused greater IgG representation observed in AuNP protein coronas. To add, Colapicchioni et al. observed similar immunoglobulin enrichment in sera obtained from pancreatic cancer patients (n = 10). HSPC:DSPG:Cholesterol liposomes were exposed to patient sera and analyzed using 1D-SDS-PAGE, with strong bands appearing at 37 & 75 kDa. Contents at these marks were isolated and identified via mass spectrometry, revealing IgA & IgG heavy chains comprising the 37 kDA band, and vitamin K, serotransferrin and albumin making up the bulk of the 75 kDa band [125]. Work performed by Caputo et al. yielded comparable results, with intense bands appearing at ~ 110, 90, 75, and 37 kDa. In their study, lipid NPs were exposed to blood samples taken from both pancreatic cancer patients (n = 20) at varying stages of disease progression and healthy controls (n = 5). Both groups displayed bands at similar locations; namely, ~ 110, 90, 75, 50 and 37 kDa. However, NPs incubated with cancer-positive sera showed significant enrichment at ~ 110, 90, 75, and 37 kDa and had greater overall protein content compared to healthy controls. Principal component analysis using band intensity predicted 5/5 controls and 17/20 cancer patients' conditions correctly, indicating that there were measurable differences in the protein levels of healthy and cancer protein coronas [126].

Increased immune protein prevalence in protein coronas was not limited to immunoglobulins. Ren et al. noted a seven to ninefold enrichment of complement proteins (particularly C1q) in protein coronas of Gd@C82(OH)22 NPs incubated in sera from lung cancer patients compared to healthy controls. C1q displayed aberrant secondary structure when bound to NPs yet was still capable of initiating classical pathway activation, which the authors noted could be exploited for future cancer immunotherapies. Notably, immunoglobulins accounted for ~ 80% of protein in protein coronas, regardless of plasma source. Nanoparticles exposed to cancer-positive sera were slightly depleted in immunoglobulins compared to controls, while displaying elevated concentrations of apolipoproteins. All protein corona characterization was performed using shotgun LC–MS/MS.

These studies collectively highlight the viability of NP-based systems for cancer diagnostics that focus on relative protein composition in protein coronas, rather than those targeting cancer-specific biomarkers. The marked upregulation of IgG, IgA, and autoantibodies seen in prostate and pancreatic cancer, as well as the notable enrichment of complement components in lung cancer protein coronas, suggests that these immune-related proteins could serve as potential indicators of cancer presence. Beyond diagnostic applications, such systems could hypothetically assess the strength of the immune responses to particular cancers and enable monitoring of immune system function over time. Additionally, relative concentrations of multiple proteins could be examined to establish disease-specific protein corona profiles, offering a novel diagnostic tool with high specificity. Such a system has been developed by Papi et al. created a pancreatic cancer-specific profile, incorporating relative

concentrations of multiple biomarkers in graphene oxide protein coronas. Using this profile, they were able to correctly predict the origin of all test samples (n = 50) with 92% sensitivity and 100% specificity [127].

Cancer-specific protein corona characterization also offers insights into improved targeted delivery, as evidenced by recent work put forward by Ezzat et al. Their group incubated folate-coated chitosan NPs in breast cancer-positive serum for 1, 12, and 24 h, revealing distinct protein corona compositions compared to healthy controls. Folate-dense NPs attracted serotransferrin, haptoglobin, and α-2 macroglobulin, with the authors proposing that the elevated serotransferrin levels could enhance NP uptake in cancer cells due to overexpression of transferrin receptors, which was previously observed in MCF-7 cell lines. Protein coronas exposed to cancer-positive sera also exhibited higher concentrations of apolipoproteins and transthyretin compared to controls. Notably, smaller NPs bound clusterin—a protein known for imparting stealth & disopsonic properties—while larger NPs did not [128]. These findings suggest that optimizing NP size and surface coatings based on disease-specific protein corona profiles could enhance targeted drug delivery. Further research is needed to develop and refine such disease-specific targeting methods for other types of cancer.

Autoimmune disease and immunomodulation related to the protein corona Disease-specific alterations in protein corona composition have been observed in autoimmune conditions as well. Notably, PEGylated polyamidoamine dendrimers showed preferential interactions (i.e. increased adsorption) for EMC8, GYS2, H2BC3, and TOLLIP proteins in synovial fluid taken from rheumatoid arthritis patients. TOM1, a protein involved in endosomal transport, was seen solely in osteoarthritis coronas, not in RA or healthy control-incubated protein coronas. The authors observed that varying degrees of PEGylation also impacted protein adsorption with COMP, ITIH1, ITIH2, and SIGLEC5 proteins observed on PEG_{350} NPs incubated in osteoarthritis & rheumatoid arthritis synovial fluid but not in PEG_{5000} NPs [129]. This work highlights the well-established phenomenon of disease-based differences in protein corona composition, as well as the role surface functionalization plays in determining such differences. Similar studies are urgently needed for other autoimmune conditions, as research in this area is broadly lacking.

While there is certainly a research gap regarding specific autoimmune diseases' impacts on protein corona composition & formation, various nanomaterials and their immunomodulatory effects have been characterized. For example, Borgognoni et al. demonstrated that TiO_2 NP exposure can induce pro- and anti-inflammatory cytokine production in macrophages. Q-TOF mass spectrometry studies revealed albumin and proteins involved in both phosphorylation and *N*- & *O*-glycosylation were overrepresented in TiO_2 protein coronas [130]. Specifically, IL-1B, IL-6, and IL-10 production was upregulated in macrophages dosed with over 10 μg/ml TiO_2 NPs. The pro-inflammatory component of this response could be due to the adsorption of proteins involved in *N*-glycosylation, a process crucial to DNA base excision repair. TiO_2 uptake may disrupt this repair mechanism, causing macrophages to release damage-associated molecular patterns (DAMPs), which, in turn, activate other macrophages and lead to cytokine production. The secretion of IL-6 is also particularly noteworthy, given its key role in the pathogenesis of autoimmune diseases. Similarly, Sumbayev et al. documented the immunomodulatory effects of AuNPs; namely, that they interfered with IL-1B signaling in macrophages, resulting in an immunosuppressed-like phenotype [131]. The authors proposed that this may be due to extracellular NP-interferon interactions, which suggests a potential protein corona-based mechanism for this finding. AuNPs have also been shown to suppress the production of both TNF-α and IL-6 in endotoxin-primed macrophages [132].

Though these specific interactions have been noted, their mechanisms (and putative mechanisms) remain largely uncharacterized. Protein corona-cytokine interactions could partially explain some of these phenomena, and thus demand further study. Further discoveries may be beneficial in developing more potent NP-based autoimmune disease treatment systems—wherein an anti-inflammatory carrier (e.x., AuNPs) is paired with an anti-inflammatory therapeutic (steroid, monoclonal antibody, etc.) to enhance efficacy. Conversely, in conditions where immune stimulation is beneficial, such as T-cell based immunotherapies, NPs could hypothetically aid in priming a host's immune response.

Considering disease-state in future nanomedicines

The impact of various diseases on protein corona composition and nanoparticle-based drug delivery systems is an evolving area of research. Other work has examined non-cancer diseases, such as diabetes and colitis [133, 134], demonstrating unique protein coronas based on disease state and a viable concern for a wide spectrum of diseases. Future studies that seek to develop NPs for specific diseases should explore alteration of the proteome and consequently protein corona formation. Investigating the variability in protein coronas across patients with the same disease, and across different disease stages, could lead to more personalized and precise diagnostic and therapeutic strategies. Further characterization of disease-specific corona profiles may enable the development

of nanoparticles optimized for improved targeting and treatment efficacy, offering a promising avenue for tailored nanomedicine. It is also important to consider multiple proteins when designing NPs for various applications as reliance on a single biomarker can lead to high rates of false positives in diagnostics and less therapeutic efficacy in targeted nanomedicines. To circumvent this issue, recent strategies have explored creating "unbiased" systems that allow for all proteins in biofluids to adsorb to the NP but also allow for adsorption for proteins that have higher affinity, namely disease-specific proteins [135].

Conclusions

Nanomedicine has the potential to address many diseases through targeted delivery of therapeutics. However, there are many patient-specific factors that are often neglected throughout the therapeutic pipeline that can affect efficacy via the NP protein corona. In this review, we summarized research of various factors that have been shown to affect the NP protein corona and serums including sex, ancestry, age, environment, and disease state. Of these factors, sex and disease state have begun to receive recognition as variables that affect protein corona. There is a pressing need to consider and establish the effects of other variables (ancestry, age, and environment) to develop high-impact nanomedicines. Notably, all the factors we have discussed have very heterogeneous behavior with respect to protein corona formation and subsequent NP behavior. For example, in human studies, there was shown to be sex-dependent uptake that was also affected by cell type [34, 42].

Notably, many of the studies discussed here regarding patient specific differences, while scientifically meaningful, use nanotechnologies that are far from use in clinic. Therefore, it is important to consider the effects of protein corona on current nanomedicines that are used clinically. All approved NPs for use in drug delivery in the clinic are PEGylated lipid-based NPs or PEG-drug conjugates [136]. The effects of protein corona on clinically relevant nanotechnologies such as PEGylated lipid NPs have been explored, but not in terms of patient specific differences. In a previously mentioned study, Poley et al. used liposomes to reveal how variations in estrous/menstrual cycles, a patient specific factor, can lead to differential therapeutic efficacy [25]. Notably, Poley et al. do not delve into how protein corona can affect NP fate which may contribute valuable insight sex-based differences. Therefore, there needs to be more research investigating these kinds of factors.

Other works have also sought to show protein corona effects in PEGylated lipid NPs. Aliakbarinodehi et al. explored how the protein corona and its modulation affects the uptake of conventional lipid NPs in Huh7 cells [137]. They found that NPs incubated with lipoprotein-rich FBS had lower adsorption of proteins at lower pH indicating that protein corona may affect endosomal escape. Conclusively, they found that lipoproteins have little effect on uptake but were suggested to inhibit endosomal escape [137]. Cheng et al. developed selective organ targeting (SORT) NPs whereby including a certain cationic lipid—a SORT molecule—diverts delivery of mRNA to spleen and lungs instead of the liver [138]. In a subsequent study, Dillard et al. postulated and proved that liver SORT NPs operate via the identical ApoE-mediated pathway as Onpattro does, but alters the composition of the protein corona upon the inclusion of SORT molecules that deliver to extrahepatic targets [139]. It was found that spleen and lung SORT NPs have coronas enriched with β2-glycoprotein I and vitronectin, respectively, both of which undergo ApoE-independent mechanisms, and improve cellular uptake and functional mRNA delivery [139]. These works emphasize important considerations in the design of nanotechnology and are complementary to each other. Poley et al. examines how patient factors affect efficacy while Cheng et al., Dilliard et al., and Aliakbarinodehi et al. highlight how different components of protein corona can affect efficacy when using clinically relevant modalities [25, 137–139].

Delivery route may also play a role in protein corona composition. Research on the topic is certainly lacking; however, it is easy to foresee that different routes would likely lead to different protein coronas. NPs in the gastrointestinal milieu face commensal microbes, digestive enzymes, and food by-products—which are not commonly seen (or seen at all) in the circulatory system [140]. NPs delivered intravenously instead would interact with complement, immunoglobulins, and albumin, among other serum proteins. Therefore, NP–based delivery systems must be designed with their targeted biological environments in mind, as different administration routes could easily alter a system's biological fate.

Future nanomedicine research must account for variations in the protein corona across different patient populations. In this review, we have highlighted how protein corona variations can cause differences in NP targeting, cellular uptake, and therapeutic cargo release rates [8]. Understanding how these factors affect the protein corona for any developed nanomedicine is essential before moving toward clinical translation. For example, Gao et al. [6] demonstrated that the protein corona formed on NPs in female fish led to preferential accumulation in the ovaries, a phenomenon not observed in male fish. This finding is critical to consider when translating NPs to clinical use. By modifying the NP surface to reduce binding affinity for these specific proteins,

accumulation in the ovaries could be minimized. Conversely, if ovarian targeting is desired, designing NPs with a stronger affinity for these proteins could offer a strategy for a sex-specific nanomedicine.

To address these challenges, researchers have developed corona-free NPs using specialized materials, and have also designed NPs that leverage the protein corona as a targeting mechanism by recruiting specific proteins into the corona [8]. Manipulating the composition and density of the protein corona with tailored materials holds significant promise for harmonizing NP behavior across diverse patient populations.

Abbreviations

AgNP	Silver nanoparticle
ARFIP2	ADP Ribosylation Factor Interacting Protein 2
AuNP	Gold nanoparticle
BA	Biological age
BMI	Body mass index
BrdU	Bromodeoxyuridine
CD40	Cluster of differentiation 40
CDH2	Cadherin-2
CXCL-10	C-X-C motif chemokine ligand 10
DDR	DNA damage response
DLS	Dynamic light scattering
DNA	Deoxyribonucleic acid
DSPG	1,2-Distearoyl-*sn*-glycero-3-[phospho-Rac-(1-glycerol)]
ECM	Extracellular matrix
eLNPs	Empty lipid nanoparticles
FGF2	Fibroblast growth factor 2
GDF15	Growth/differentiation factor 15
GIT	Gastrointestinal tract
GLM	Generalized linear model
hAMSC	Human amniotic stem cell
HSPC	Hydrogenated Soy Phosphatidylcholine
HTCP	High-throughput cell phenotyping
IFNγ	Interferon-gamma
IgG	Immunoglobulin g
kDa	Kilodalton
LC–MS	Liquid chromatography mass spectrometry
NHDF	Normal human dermal fibroblasts
NK	Natural killer
NP	Nanoparticle
OA	Osteoarthritis
OAS1	2′-5′ Oligoadenylate synthetase
PAI	Plasminogen activator inhibitor
PEG	Polyethylene glycol
pQTL	Protein quantitative trait loci
PTSD	Post-traumatic stress disorder
PVP-AgNP	Polyvinylpyrrolidone-coated silver nanoparticles
QD	Quantum dot
RA	Rheumatoid arthritis
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SGF	Stimulated gastric fluid
SIF	Simulated intestinal fluid
SiNPs	Silica NPs
SORT	Selective organ targeting
TiO_2 NPs	Titanium dioxide nanoparticles
UV	Ultraviolet
ZP	Zona pellucida

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