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The nano-plasma interface: implications of the protein corona

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Abstract

The interactions between nanoparticles and macromolecules in the blood plasma dictate the biocompatibility and efficacy of nanotherapeutics. Accordingly, the properties of nanoparticles and endogenous biomolecules change at the nano-plasma interface. Here, we review the implications of such changes including toxicity, immunological recognition, molecular targeting, biodistribution, intracellular uptake, and drug release. Although this interface poses several challenges for nanomedicine, it also presents opportunities for exploiting nanoparticle-protein interactions.

Keywords

nanoparticles; protein corona; nano-bio interface; proteins; plasma

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1. Introduction

Nanoparticles present promising tools for diagnostic and therapeutic purposes. Accordingly, the scientific literature is replete with examples of nanosystems designed for medical applications [1–6]. Moreover, several nanotherapeutics have already received clinical approval and many are currently undergoing clinical trials [7]. These nanoparticles are intended for either local or systemic administration. The latter route provides a means for targeting tissue that is inaccessible through local infusion, making it a suitable method for treating diseases such as metastatic cancer. Upon systemic injection, nanoparticles are subjected to a variety of forces and biological reactions in the blood. These phenomena include, but are not limited to, mechanical stress due to rapid blood flow, enzymatic degradation, binding to biomolecules, and uptake by immune cells [8]. This review will focus specifically on the interactions between nanoparticles and components of blood plasma. Since the blood contains thousands of proteins [9], it is not surprising that such interactions occur. Notably, the nano-plasma interface may influence both the nanoparticles and the biomolecules that they come in contact with. In essence, the characteristics of nanoparticles as well as plasma components could markedly change at the interface. These interactions may lower or increase the toxicity of nanosystems and in turn change the biodistribution and efficacy of nanotherapeutics. Therefore, there has recently been an impetus towards understanding the impact of blood molecules on nanostructures and vice versa. Gaining a better understanding of the nano-plasma interface could aid in overcoming challenges in nanomedicine and provide opportunities for exploiting these kinds of interactions.

2. The protein corona

The biomolecule coating that forms around nanoparticles upon contact with biological fluids is termed a protein corona. The corona forms due to the high surface free energy of nanoparticles, resulting in absorption of various molecules, most notably proteins. The binding forces that are responsible for such interactions include van der Waals interactions, hydrogen bonds, hydrophobic interactions, electrostatic interactions, and π- π stacking [10]. Indeed, protein shells have been reported to form around a vast array of nanoparticles, including those comprised of metal [11–13], polystyrene [14–16], silica [14–17] and, lipids [18]. The current hypothesis states that the corona consists of a ‘hard’ and ‘soft’ layer (Fig. 1) [19]. These components are distinguishable in how tightly the biomolecules are associated with the nanoparticles. In addition, the soft layer is thought to be more dynamic, consisting of rapidly exchanging biomolecules. However, there exists controversy regarding the heterogeneity of the hard corona that forms in human blood plasma. In this regard, various sources report the presence of less than 100 different proteins [20–22], while others claim the existence of more than100 [16, 23]. Notably, this disparity may be due to the varying nature of the nanoparticles studied or differences in the methodological approaches used to identify protein profiles. In general, the most abundant proteins in the corona are albumin, apolipoprotein-1, complement proteins, and immunoglobulins [16, 20, 21, 23].

The composition of the protein corona varies depending on the nanoparticle material, size, shape and surface charge [22, 24]. For instance, hydrophobic nanoparticles, e.g. carbon

nanotubes, usually attract proteins with several hydrophobic residues [25]. In addition, enhanced protein absorption is observed with increased nanoparticle size [26]. This size-dependent effect can be explained by taking into account the curvature of nanoparticles. Larger nanoparticles have reduced curvature, which enables proteins to more freely interact with a larger surface area. The shape of nanoparticles also governs the type of protein corona that forms. Notable, titanium dioxide nanotubes and nanorods display different protein corona characteristics [27]. In addition, a positive surface charge is typically correlated with increased protein absorption [28]. Moreover, while there exists a consensus concerning the dynamic nature of the protein corona, the sequential binding events at the nano-plasma interface are largely unknown. Interestingly, a recent study found that the amount of protein in the corona changes over time, while the types of bound protein remain relatively constant [16].

3. Changes to biomolecules at the nano-plasma interface

When proteins bind to nanoparticles they may undergo conformational changes. Such changes can be either reversible or irreversible. For instance, the absorption of albumin on the surface of gold nanoparticles changes the secondary and tertiary conformation of this protein [29]. In addition, intracellular proteins, such as cytochrome c [30] and ribonuclease A [31] have also been found to undergo structural changes when exposed to nanoparticles. Notably, a correlation between nanoparticle size and protein unfolding has been observed. Larger nanoparticles with lower surface curvature cause more conformational changes in protein structure [32–34]. Since the structure-function relationship is strong for proteins, nanoparticle coronas may also alter the behavior of these macromolecules. As an illustration, iron oxide nanoparticles were found to change the conformation of transferrin, causing the protein to prematurely release iron [35]. Moreover, the conformational alteration is irreversible, indicating permanent damage to the function of this protein in iron transport. In addition, as proteins come in close vicinity to each other at the nanoparticle surface, they may cluster together. Indeed, one study found that the fibrillation of human β2-microglobulin increases as a result of exposure to several different nanoparticles [36].

4. Changes to nanoparticles at the nano-plasma interface

The characteristics of nanoparticles tend to change considerably upon interactions with a biological environment. In particular, properties such as the shape, size, and charge are usually affected as a consequence of the protein corona. In general, nanoparticles become larger as a result of protein interactions. This size increase is usually in the range of 20–70 nm [14, 16, 17, 37], suggesting that the corona consist of multiple protein layers. However, the parameters that dictate the number of layers surrounding a nanoparticle remain elusive. In contrast, the size of certain lipid nanoparticles decreases in a protein-rich environment, presumably due to osmotic forces [38]. Namely, as the lipid membrane is impermeable to proteins, an osmotic pressure is created at the nano-plasma interface. Consequently, water flows out of the aqueous interior, causing nanoparticle compression. Furthermore, the protein corona may trigger nanoparticle aggregation through protein bridges, resulting in the formation of larger clusters [27, 39, 40]. Conversely, the presence of a protein corona may also stabilize particles and prevent aggregation [41, 42]. This stabilization effect may arise

due to changes in the nanoparticle surface charge or steric hindrance of inter-particle binding [42].

In addition, as most proteins are negatively charged, the formation of a protein corona will cause the nanoparticle surface to become anionic. One study showed that regardless of the zeta potential of bare nanoparticles (ranging from −28 mV to 51 mV), upon exposure to plasma proteins the zeta potential become negative (ranging from −24 mV to −6 mV) [16]. Although certain trends are evident regarding protein-induced changes in nanoparticle characteristics, it is difficult to form general rules about the behavior of particles in plasma. The vast array of nanoparticles and experimental conditions used to study the nano-plasma interface inevitably lead to variable conclusions regarding protein-particle interactions.

5. Implications of the nano-plasma interface

As discussed in the previous sections, the properties of nanoparticles and endogenous macromolecules change at the nano-plasma interface. Such changes may impact the efficacy and biocompatibility of nanotherapeutics. Consequently, the development of successful nanodrugs necessitates an increased understanding of the nanoparticle-corona complex. The following section will discuss some of the most important implications of protein-particle interactions (Table 1).

5.1. Toxicity

It is important to evaluate the safety of nanomaterials, as they have unique properties that are distinct from bulk materials [43–47]. The safety assessment of nanoparticles should be done on the molecular, cellular and organ level. Notably, the interactions between plasma components and nanoparticles may have implications for nanotoxicology. In fact, the nano-plasma interface can trigger toxicity due to modification of endogenous proteins [48, 49]. Such modifications can disrupt the normal function of proteins and potentially cause complications, since misfolded proteins have been associated with various diseases [50]. For instance, nanoparticle-induced conformational changes in fibrinogen were shown to activate inflammatory signaling pathways [51]. On the contrary, the formation of a protein corona can also mitigate the cytotoxicity of nanoparticles. Especially in the case of nanoparticle-cell membrane interactions, the presence of a protein coat can decrease cell damage [25, 52, 53]. Indeed, nanoparticles that are not pre-coated with a protein layer may instead interact with cell membrane proteins, causing damage to these structures. For instance, it has been shown that the toxicity of carbon nanotubes [25] and gold nanorods [54] decreases upon coating with plasma proteins. A positive correlation also exists between protein density and decreased toxicity [25]. In addition, whereas pristine silica nanoparticles have been shown to activate thrombocytes and cause hemolysis, these adverse events can be prevented in the presence of a protein coating [16]. In conclusion, the protein corona can reduce the overall toxicity of nanoparticles, while simultaneously impairing the function of a subset of plasma proteins.

5.2. Immunological recognition

A major biological barrier for the successful delivery of nanotherapeutics is the reticuloendothelial system (RES) [2]. Upon entering the body, nanoparticles are usually engulfed by immune cells in the filtrating organs of the RES. Thus, the major portion of systemically injected nanoparticles accumulates in the liver and spleen, rather than in the intended target tissue [55, 56]. Notably, immunological recognition takes place through binding of immunostimulatory proteins to the nanoparticle surface, a process termed opsonization [57]. Examples of such proteins are the immunoglobulins, which are among the most abundant macromolecules in the protein corona (see section 2). Immunoglobulins can bind directly to phagocytic cells or indirectly stimulate phagocytosis through activation of the complement system [58]. Indeed, macrophages, neutrophils, and dendritic cells express Fc receptors, which can bind to opsonins on the nanoparticle surface, thereby inducing engulfment [58]. In addition to receptor activation, nanoparticles can trigger immunological recognition through non-specific interactions between surface bound opsonins and immune cells [57]. Furthermore, it is possible that nanoparticle-induced conformational protein alterations may activate the immune system, since the body no longer recognizes the proteins as native biomolecules. Accordingly, one study speculated that the complement C3 protein may bind to misfolded albumin on the nanoparticle surface, thereby triggering an immune response [59]. In exceptional cases, nanomaterials may by themselves bind to immune receptors, causing immunostimulation [60].

In order to prevent immunological recognition, several antifouling agents have been developed, which reduce protein binding through the formation of a steric barrier around the nanoparticle. For instance, polyethylene glycol (PEG) has frequently been incorporated into various nanodelivery systems [4, 61–63]. However, the presence of stealth polymers does not completely prevent protein binding [64, 65]. In particular, the density and shape of PEG chains on a liposomal surface governs the degree of protein interactions [66]. Sparsely placed PEG chains tend to display a mushroom conformation, while densely positioned chains exhibit a brush structure (Fig. 2). The brush conformation is more effective at protecting the nanoparticle from protein binding. Nevertheless, upon repeated administration of pegylated nanotherapeutics the body may develop antibodies against PEG [67]. This effect, termed the accelerated blood clearance (ABC) phenomenon, paradoxically causes an increased immunological clearance of pegylated particles.

5.3. Molecular targeting

Over the past few decades, a plethora of targeted nanoparticles has been developed. Unfortunately, the benefits of molecular targeting agents have not been as promising as initially expected [68] and to date there are no targeted nanotherapeutics on the market. A potential reason for the limited success of targeted nanosystems is the structural modification and masking of nanoparticle surface moieties, due to the formation of a protein corona in the blood circulation [69]. As an illustration of this phenomenon, one study demonstrated that the targeting ability of transferrin-coated nanoparticles decreases as the concentration of serum in the media increases [70]. In addition, other studies have shown that coating nanoparticles with tumor-specific antibodies does not enhance tumor accumulation *in vivo* [71, 72]. Notably, two of the three studies incorporated PEG in the

nanodelivery system. Although pegylation may reduce protein binding, it may also mask or change the orientation of surface ligands. Indeed, increased PEG density on nanoparticles has previously been correlated to decreased targeting capability [73].

5.4. Biodistribution

An emerging idea is that the protein corona dictates the transport properties of nanoparticles, thereby bestowing a biological identity upon them [19, 74]. In essence, the protein coating governs the nanoparticle biodistribution, as plasma proteins exhibit distinct affinities for different tissues. As an illustration, opsonins induce liver and spleen accumulation [57], while apolipoproteins cause nanoparticle deposition in the brain [75]. In addition, albumin can reduce liver deposition and prolong blood circulation times [76]. Tumors also exhibit increased uptake of albumin, due to the enhanced permeability and retention (EPR) effect, and receptor-mediated endothelial transcytosis of the protein [77]. This phenomenon has been exploited for the development of albumin-bound therapeutics for cancer therapy [77].

Furthermore, protein-induced changes in nanoparticle size could impact biodistribution. The size of nanoscale objects is a determining factor for their localization in the body. For instance, particles smaller than 5 nm are excreted by the kidneys [78], while particles larger than 100 nm increasingly accumulate in the liver [79]. In addition, diseases, such as pancreatic cancer, which are characterized by hypovascularity and hypopermeability, require treatment with particles smaller than 50 nm [79]. Overall, such size-dependent effects on nanoparticle biodistribution suggest that nanoparticles should regularly be characterized in the presence of plasma proteins. As a hypothetical example, a 30 nm nanoparticle is selected for the treatment of pancreatic cancer due to its small size, which has been determined in a buffer solution. However, upon entering the blood circulation, the nanoparticle size increases to 80 nm, making it impermeable to the vasculature of pancreatic tumors. This example demonstrates how the characterization of a bare material may be of little use for the design of successful nanotherapeutics.

5.5. Intracellular uptake

The presence of a protein corona can impact cellular uptake of nanoparticles. In most cases, a protein coating has been shown to decrease adhesion of nanoparticles to the cell membrane, thereby reducing cellular internalization [53, 76, 80–82]. One study hypothesized that serum proteins interfere with cell membrane interactions that are required for scavenger receptor-mediated uptake of DNA-gold nanoparticles [82]. Accordingly, a correlation has been found between the amount of proteins covering the nanoparticle and the degree of cellular uptake [83]. On the contrary, other studies have shown that the uptake of particles increases in the presence of a protein coating [16, 84, 85]. Moreover, the protein corona can change the mechanism of cellular internalization. It has been demonstrated that the DNA transfection efficacy of cationic liposomes increased when they were coated with serum proteins [86]. The authors speculated that the protein interactions cause a change in the route of cellular uptake, from clathrin to caveolae-mediated endocytocis, thereby affecting the efficiency of DNA delivery. Although this change was attributed to a size-dependent effect caused by protein-induced nanoparticle aggregation, it serves to illustrate the impact of a protein corona on nanoparticle interactions with the cellular machinery.

5.6. Drug release

There are several ways in which protein-nanoparticle interactions may influence drug release. As the characteristics of nanoparticles change (e.g. size, charge and shape), drug release may be impeded or accelerated. For instance, liposomal particles that are subject to shrinkage due to osmotic forces could potentially undergo a burst-release effect when entering the blood [38]. In essence, therapeutics that are embedded in the aquatic core may be released as water is forced out of the nanoparticle. Similarly, hydrophobic agents in the bilayer could be released as the lipids compress upon liposomal shrinkage. In addition, serum-induced destabilization of particles may trigger premature drug release. As a counterexample, the binding of proteins to porous silicon particles was shown to delay drug release [87]. Namely, the absorbed proteins clogged the pores of the particle, preventing the release of the entrapped drug.

4. Opportunities

Although the nano-plasma interface poses several problems for nanodelivery systems, an increased understanding of nanoparticle-protein interactions may provide new opportunities for exploiting these phenomena. For instance, the proteins that bind to the surface of nanoparticles could provide a fingerprint of the biological milieus that the particles have been exposed to. Indeed, as nanoparticles travel through different bodily fluids the protein corona changes progressively [88]. However, a portion of the proteins remain attached to the surface, providing a molecular signature of the sequential environments that the particles have passed through [88]. This signature could be used to study the transport pathways of nanoparticles.

Moreover, the blood encompasses several endogenous transport mechanisms for the delivery of hormones, nutrients, and other macromolecules to specific tissues. The plasma proteins involved in these transport routes could be exploited for tissue-specific delivery of nanoparticles. Through increased comprehension of the interactions at the nano-plasma interface, the surface of nanoparticles could be designed to favor the binding of certain plasma proteins [69]. Accordingly, a systematic analysis of the protein binding properties of different nanomaterials, surfactant coatings, and surface modifications could prove useful in identifying nanoparticles that have increased affinity for plasma proteins of interest. Such strategies would enable some control over protein binding, thereby providing opportunities for improving the biodistribution, biocompatibility and efficacy of nanoparticles. For example, coating of nanoparticles with polysorbate 80 was shown to cause preferential binding of the plasma protein apolipoprotein E, which in turn enhanced nanoparticle accumulation in the brain [75]. Notably, this surface-engineering strategy is equally effective as the conjugation of apolipoprotein E to the nanoparticle prior to systemic administration. Similarly, upon exposure to human plasma, cationic lipid/DNA particles displayed a protein corona dominated by vitronectin [89]. Consequently, these nanoparticles had increased uptake in cancer cells expressing receptors for vitronectin.

Furthermore, as nanoparticles with cell-specific molecular targeting agents have had limited success, an alternative approach involving the use of molecular moieties for targeting plasma proteins could prove more useful. Since these moieties would already make contact

with their respective targets during the formation of a protein corona, this approach may be more effective than conventional targeting, which occurs after a protein coating has formed (Fig. 3). However, the targeted plasma protein should be abundant enough to cover the nanoparticle surface, thereby limiting space for other proteins to bind. Moreover, the protein epitopes that are important for cellular recognition should remain functional when bound to the nanoparticle. Alternatively, the composition of the protein corona could be controlled by the use of nanomaterials that form binding pockets for specific plasma proteins. For instance, nanoparticle-polymer constructs that recognize riboflavin, L-thyroxine or oestradiol were identified by screening a library of small molecules [90]. Notably, the polymers only have affinity for these molecules when constrained onto carbon nanotubes. An interesting application of these nanocarriers is the detection of biomarkers in the body, which can be achieved through incorporation of fluorescent constructs [90].

5. Conclusion

The formation of a protein corona influences the characteristics of proteins and nanoparticles. Indeed, proteins that interact with nanoparticles may undergo structural modifications that could impair their biological activity. Such alterations to the structure and function of native proteins could potentially trigger adverse effects. Likewise, the properties of nanoparticles usually change dramatically at the nano-plasma interface. For instance, an alteration to the size, shape or charge will change the biological identity of the nanoparticle. Consequently, the protein coating will impact nanoparticle toxicity, immunological recognition, targeting capability, biodistribution, intracellular uptake and drug release. Therefore, nanoparticles should be characterized in environments that replicate *in vivo* conditions. Accordingly, an increased understanding of nanoparticle-protein interactions will be essential for the design of biocompatible and efficacious nanotherapeutics. Furthermore, the nano-plamsa interface may provide unique opportunities for controlling nanoparticle accumulation, through utilization of endogenous transport mechanisms.

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References

1. Blanco E, Hsiao A, Mann AP, Landry MG, Meric-Bernstam F, Ferrari M. Nanomedicine in cancer therapy: innovative trends and prospects. Cancer science. 2011; 102:1247–1252. [PubMed: 21447010]
2. Ferrari M. Cancer nanotechnology: opportunities and challenges. Nature reviews. Cancer. 2005; 5:161–171.
3. Gentile E, Cilurzo F, Di Marzio L, Carafa M, Ventura CA, Wolfram J, Paolino D, Celia C. Liposomal chemotherapeutics. Future Oncology. 2013; 9:1849–1859. [PubMed: 24295415]
4. Molinaro R, Wolfram J, Federico C, Cilurzo F, Di Marzio L, Ventura CA, Carafa M, Celia C, Fresta M. Polyethylenimine and chitosan carriers for the delivery of RNA interference effectors. Expert opinion on drug delivery. 2013; 10:1653–1668. [PubMed: 24090239]

5. Shen J, Xu R, Mai J, Kim HC, Guo X, Qin G, Yang Y, Wolfram J, Mu C, Xia X, Gu J, Liu X, Mao ZW, Ferrari M, Shen H. High capacity nanoporous silicon carrier for systemic delivery of gene silencing therapeutics. ACS nano. 2013; 7:9867–9880. [PubMed: 24131405]
6. Ma X, Zhao Y, Liang XJ. Theranostic nanoparticles engineered for clinic and pharmaceutics. Accounts of chemical research. 2011; 44:1114–1122. [PubMed: 21732606]
7. Davis ME, Chen ZG, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. Nature reviews. Drug discovery. 2008; 7:771–782.
8. Ferrari M. Frontiers in cancer nanomedicine: directing mass transport through biological barriers. Trends in biotechnology. 2010; 28:181–188. [PubMed: 20079548]
9. Farrah T, Deutsch EW, Omenn GS, Campbell DS, Sun Z, Bletz JA, Mallick P, Katz JE, Malmstrom J, Ossola R, Watts JD, Lin B, Zhang H, Moritz RL, Aebersold R. A high-confidence human plasma proteome reference set with estimated concentrations in PeptideAtlas. Molecular & cellular proteomics : MCP. 2011; 10 M110 006353.
10. Yang ST, Liu Y, Wang YW, Cao A. Biosafety and bioapplication of nanomaterials by designing protein-nanoparticle interactions. Small. 2013; 9:1635–1653. [PubMed: 23341247]
11. Shannahan JH, Lai X, Ke PC, Podila R, Brown JM, Witzmann FA. Silver nanoparticle protein corona composition in cell culture media. PloS one. 2013; 8:e74001. [PubMed: 24040142]
12. Casals E, Pfaller T, Duschl A, Oostingh GJ, Puntes VF. Hardening of the nanoparticle-protein corona in metal (Au, Ag) and oxide (Fe3O4, CoO, and CeO2) nanoparticles. Small. 2011; 7:3479–3486. [PubMed: 22058075]
13. Casals E, Pfaller T, Duschl A, Oostingh GJ, Puntes V. Time evolution of the nanoparticle protein corona. ACS nano. 2010; 4:3623–3632. [PubMed: 20553005]
14. Monopoli MP, Walczyk D, Campbell A, Elia G, Lynch I, Bombelli FB, Dawson KA. Physical-chemical aspects of protein corona: relevance to in vitro and in vivo biological impacts of nanoparticles. Journal of the American Chemical Society. 2011; 133:2525–2534. [PubMed: 21288025]
15. Walczyk D, Bombelli FB, Monopoli MP, Lynch I, Dawson KA. What the cell "sees" in bionanoscience. Journal of the American Chemical Society. 2010; 132:5761–5768. [PubMed: 20356039]
16. Tenzer S, Docter D, Kuharev J, Musyanovych A, Fetz V, Hecht R, Schlenk F, Fischer D, Kiouptsi K, Reinhardt C, Landfester K, Schild H, Maskos M, Knauer SK, Stauber RH. Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. Nature nanotechnology. 2013; 8:772–781.
17. Mortensen NP, Hurst GB, Wang W, Foster CM, Nallathamby PD, Retterer ST. Dynamic development of the protein corona on silica nanoparticles: composition and role in toxicity. Nanoscale. 2013; 5:6372–6380. [PubMed: 23736871]
18. Barran-Berdon AL, Pozzi D, Caracciolo G, Capriotti AL, Caruso G, Cavaliere C, Riccioli A, Palchetti S, Lagana A. Time evolution of nanoparticle-protein corona in human plasma: relevance for targeted drug delivery. Langmuir : the ACS journal of surfaces and colloids. 2013; 29:6485–6494. [PubMed: 23631648]
19. Monopoli MP, Aberg C, Salvati A, Dawson KA. Biomolecular coronas provide the biological identity of nanosized materials. Nature nanotechnology. 2012; 7:779–786.
20. Dobrovolskaia MA, Patri AK, Zheng J, Clogston JD, Ayub N, Aggarwal P, Neun BW, Hall JB, McNeil SE. Interaction of colloidal gold nanoparticles with human blood: effects on particle size and analysis of plasma protein binding profiles. Nanomedicine : nanotechnology, biology, and medicine. 2009; 5:106–117.
21. Martel J, Young D, Young A, Wu CY, Chen CD, Yu JS, Young JD. Comprehensive proteomic analysis of mineral nanoparticles derived from human body fluids and analyzed by liquid chromatography-tandem mass spectrometry. Analytical biochemistry. 2011; 418:111–125. [PubMed: 21741946]
22. Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA. Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:14265–14270. [PubMed: 18809927]

23. Tenzer S, Docter D, Rosfa S, Wlodarski A, Kuharev J, Rekik A, Knauer SK, Bantz C, Nawroth T, Bier C, Sirirattanapan J, Mann W, Treuel L, Zellner R, Maskos M, Schild H, Stauber RH. Nanoparticle size is a critical physicochemical determinant of the human blood plasma corona: a comprehensive quantitative proteomic analysis. ACS nano. 2011; 5:7155–7167. [PubMed: 21866933]
24. Zhu M, Nie G, Meng H, Xia T, Nel A, Zhao Y. Physicochemical properties determine nanomaterial cellular uptake, transport, and fate. Accounts of chemical research. 2013; 46:622–631. [PubMed: 22891796]
25. Ge C, Du J, Zhao L, Wang L, Liu Y, Li D, Yang Y, Zhou R, Zhao Y, Chai Z, Chen C. Binding of blood proteins to carbon nanotubes reduces cytotoxicity. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:16968–16973. [PubMed: 21969544]
26. Cedervall T, Lynch I, Lindman S, Berggard T, Thulin E, Nilsson H, Dawson KA, Linse S. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:2050–2055. [PubMed: 17267609]
27. Deng ZJ, Liang M, Toth I, Monteiro MJ, Minchin RF. Molecular interaction of poly(acrylic acid) gold nanoparticles with human fibrinogen. ACS nano. 2012; 6:8962–8969. [PubMed: 22998416]
28. Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. Molecular pharmaceutics. 2008; 5:505–515. [PubMed: 18672949]
29. Shang L, Wang Y, Jiang J, Dong S. pH-dependent protein conformational changes in albumin:gold nanoparticle bioconjugates: a spectroscopic study. Langmuir : the ACS journal of surfaces and colloids. 2007; 23:2714–2721. [PubMed: 17249699]
30. Shang W, Nuffer JH, Muniz-Papandrea VA, Colon W, Siegel RW, Dordick JS. Cytochrome C on silica nanoparticles: influence of nanoparticle size on protein structure, stability, and activity. Small. 2009; 5:470–476. [PubMed: 19189325]
31. Shang W, Nuffer JH, Dordick JS, Siegel RW. Unfolding of ribonuclease A on silica nanoparticle surfaces. Nano letters. 2007; 7:1991–1995. [PubMed: 17559285]
32. Teichroeb JH, Forrest JA, Jones LW. Size-dependent denaturing kinetics of bovine serum albumin adsorbed onto gold nanospheres. The European physical journal. E, Soft matter. 2008; 26:411–415.
33. Teichroeb JH, Forrest JA, Ngai V, Jones LW. Anomalous thermal denaturing of proteins adsorbed to nanoparticles. The European physical journal. E, Soft matter. 2006; 21:19–24.
34. Lundqvist M, Sethson I, Jonsson BH. Protein adsorption onto silica nanoparticles: conformational changes depend on the particles' curvature and the protein stability. Langmuir : the ACS journal of surfaces and colloids. 2004; 20:10639–10647. [PubMed: 15544396]
35. Mahmoudi M, Shokrgozar MA, Sardari S, Moghadam MK, Vali H, Laurent S, Stroeve P. Irreversible changes in protein conformation due to interaction with superparamagnetic iron oxide nanoparticles. Nanoscale. 2011; 3:1127–1138. [PubMed: 21210042]
36. Linse S, Cabaleiro-Lago C, Xue WF, Lynch I, Lindman S, Thulin E, Radford SE, Dawson KA. Nucleation of protein fibrillation by nanoparticles. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:8691–8696. [PubMed: 17485668]
37. Goy-Lopez S, Juarez J, Alatorre-Meda M, Casals E, Puntes VF, Taboada P, Mosquera V. Physicochemical characteristics of protein-NP bioconjugates: the role of particle curvature and solution conditions on human serum albumin conformation and fibrillogenesis inhibition. Langmuir : the ACS journal of surfaces and colloids. 2012; 28:9113–9126. [PubMed: 22439664]
38. Wolfram J, Suri K, Celia C, Yang Y, Shen J, Fresta M, Zhao Y, Shen H, Ferrari M. Shrinkage of pegylated and non-pegylated liposomes in serum. Colloids and surfaces. B, Biointerfaces. 2013; 114:294–300.
39. Zhu J, Sun Z, Li JJ, Zhao JW. Bovine serum albumins (BSA) induced aggregation and separation of gold colloid nanoparticles. Journal of nanoscience and nanotechnology. 2012; 12:2206–2211. [PubMed: 22755039]

40. Kendall M, Ding P, Kendall K. Particle and nanoparticle interactions with fibrinogen: the importance of aggregation in nanotoxicology. Nanotoxicology. 2011; 5:55–65. [PubMed: 21417688]
41. Dominguez-Medina S, Blankenburg J, Olson J, Landes CF, Link S. Adsorption of a Protein Monolayer via Hydrophobic Interactions Prevents Nanoparticle Aggregation under Harsh Environmental Conditions. ACS sustainable chemistry & engineering. 2013; 1:833–842. [PubMed: 23914342]
42. Wells MA, Abid A, Kennedy IM, Barakat AI. Serum proteins prevent aggregation of Fe2O3 and ZnO nanoparticles. Nanotoxicology. 2012; 6:837–846. [PubMed: 22149273]
43. Nel A, Zhao Y, Madler L. Environmental health and safety considerations for nanotechnology. Accounts of chemical research. 2013; 46:605–606. [PubMed: 23964654]
44. Zhao Y, Nel A, Riehemann K. Filling knowledge gaps that distinguish the safety profiles of nano versus bulk materials. Small. 2013; 9:1426–1427. [PubMed: 23681784]
45. Zhao F, Zhao Y, Liu Y, Chang X, Chen C, Zhao Y. Cellular uptake, intracellular trafficking, and cytotoxicity of nanomaterials. Small. 2011; 7:1322–1337. [PubMed: 21520409]
46. Zhang P, Ma Y, Zhang Z, He X, Guo Z, Tai R, Ding Y, Zhao Y, Chai Z. Comparative toxicity of nanoparticulate/bulk Yb(2)O(3) and YbCl(3) to cucumber (Cucumis sativus). Environmental science & technology. 2012; 46:1834–1841. [PubMed: 22191482]
47. Chen Z, Meng H, Xing GM, Chen CY, Zhao YL. Toxicological and biological effects of nanomaterials. Int J Nanotechnol. 2007; 4:179–196.
48. Zuo G, Kang SG, Xiu P, Zhao Y, Zhou R. Interactions between proteins and carbon-based nanoparticles: exploring the origin of nanotoxicity at the molecular level. Small. 2013; 9:1546–1556. [PubMed: 23038664]
49. Pelaz B, Charron G, Pfeiffer C, Zhao Y, de la Fuente JM, Liang XJ, Parak WJ, Del Pino P. Interfacing engineered nanoparticles with biological systems: anticipating adverse nano-bio interactions. Small. 2013; 9:1573–1584. [PubMed: 23112130]
50. Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. Annual review of biochemistry. 2006; 75:333–366.
51. Deng ZJ, Liang M, Monteiro M, Toth I, Minchin RF. Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation. Nature nanotechnology. 2011; 6:39–44.
52. Hu W, Peng C, Lv M, Li X, Zhang Y, Chen N, Fan C, Huang Q. Protein corona-mediated mitigation of cytotoxicity of graphene oxide. ACS nano. 2011; 5:3693–3700. [PubMed: 21500856]
53. Lesniak A, Fenaroli F, Monopoli MP, Aberg C, Dawson KA, Salvati A. Effects of the presence or absence of a protein corona on silica nanoparticle uptake and impact on cells. ACS nano. 2012; 6:5845–5857. [PubMed: 22721453]
54. Wang L, Li J, Pan J, Jiang X, Ji Y, Li Y, Qu Y, Zhao Y, Wu X, Chen C. Revealing the binding structure of the protein corona on gold nanorods using synchrotron radiation-based techniques: understanding the reduced damage in cell membranes. Journal of the American Chemical Society. 2013; 135:17359–17368. [PubMed: 24215358]
55. Kumar R, Roy I, Ohulchanskky TY, Vathy LA, Bergey EJ, Sajjad M, Prasad PN. In vivo biodistribution and clearance studies using multimodal organically modified silica nanoparticles. ACS nano. 2010; 4:699–708. [PubMed: 20088598]
56. Lee MJ, Veiseh O, Bhattarai N, Sun C, Hansen SJ, Ditzler S, Knoblaugh S, Lee D, Ellenbogen R, Zhang M, Olson JM. Rapid pharmacokinetic and biodistribution studies using cholorotoxin-conjugated iron oxide nanoparticles: a novel non-radioactive method. PloS one. 2010; 5:e9536. [PubMed: 20209054]
57. Owens DE 3rd, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. International journal of pharmaceutics. 2006; 307:93–102. [PubMed: 16303268]
58. Goodridge HS, Underhill DM, Touret N. Mechanisms of Fc receptor and dectin-1 activation for phagocytosis. Traffic. 2012; 13:1062–1071. [PubMed: 22624959]
59. Vauthier C, Persson B, Lindner P, Cabane B. Protein adsorption and complement activation for di-block copolymer nanoparticles. Biomaterials. 2011; 32:1646–1656. [PubMed: 21093043]

60. Yang Y, Wolfram J, Fang X, Shen H, Ferrari M. Polyarginine Induces an Antitumor Immune Response through Binding to Toll-Like Receptor 4. Small. 2013
61. Schipper ML, Iyer G, Koh AL, Cheng Z, Ebenstein Y, Aharoni A, Keren S, Bentolila LA, Li J, Rao J, Chen X, Banin U, Wu AM, Sinclair R, Weiss S, Gambhir SS. Particle size, surface coating, and PEGylation influence the biodistribution of quantum dots in living mice. Small. 2009; 5:126–134. [PubMed: 19051182]
62. Celia C, Trapasso E, Locatelli M, Navarra M, Ventura CA, Wolfram J, Carafa M, Morittu VM, Britti D, Di Marzio L, Paolino D. Anticancer activity of liposomal bergamot essential oil (BEO) on human neuroblastoma cells. Colloids and surfaces. B, Biointerfaces. 2013; 112:548–553.
63. Wolfram J, Suri K, Huang Y, Molinaro R, Borsoi C, Scott B, Boom K, Paolino D, Fresta M, Wang J, Ferrari M, Celia C, Shen H. Evaluation of anticancer activity of celastrol liposomes in prostate cancer cells. Journal of Microencapsulation. 2014
64. Hamad I, Al-Hanbali O, Hunter AC, Rutt KJ, Andresen TL, Moghimi SM. Distinct polymer architecture mediates switching of complement activation pathways at the nanosphere-serum interface: implications for stealth nanoparticle engineering. ACS nano. 2010; 4:6629–6638. [PubMed: 21028845]
65. Kim HR, Andrieux K, Delomenie C, Chacun H, Appel M, Desmaele D, Taran F, Georgin D, Couvreur P, Taverna M. Analysis of plasma protein adsorption onto PEGylated nanoparticles by complementary methods: 2-DE, CE and Protein Lab-on-chip system. Electrophoresis. 2007; 28:2252–2261. [PubMed: 17557357]
66. Bartucci R, Pantusa M, Marsh D, Sportelli L. Interaction of human serum albumin with membranes containing polymer-grafted lipids: spin-label ESR studies in the mushroom and brush regimes. Biochimica et biophysica acta. 2002; 1564:237–242. [PubMed: 12101018]
67. Knop K, Hoogenboom R, Fischer D, Schubert US. Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. Angew Chem Int Ed Engl. 2010; 49:6288–6308. [PubMed: 20648499]
68. Kwon IK, Lee SC, Han B, Park K. Analysis on the current status of targeted drug delivery to tumors. Journal of controlled release : official journal of the Controlled Release Society. 2012; 164:108–114. [PubMed: 22800574]
69. Mahon E, Salvati A, Baldelli Bombelli F, Lynch I, Dawson KA. Designing the nanoparticle-biomolecule interface for "targeting and therapeutic delivery". Journal of controlled release : official journal of the Controlled Release Society. 2012; 161:164–174. [PubMed: 22516097]
70. Salvati A, Pitek AS, Monopoli MP, Prapainop K, Bombelli FB, Hristov DR, Kelly PM, Aberg C, Mahon E, Dawson KA. Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. Nature nanotechnology. 2013; 8:137–143.
71. Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB, Marks JD, Benz CC, Park JW. Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. Cancer research. 2006; 66:6732–6740. [PubMed: 16818648]
72. Cirstoiu-Hapca A, Buchegger F, Lange N, Bossy L, Gurny R, Delie F. Benefit of anti-HER2-coated paclitaxel-loaded immuno-nanoparticles in the treatment of disseminated ovarian cancer: Therapeutic efficacy and biodistribution in mice. Journal of controlled release : official journal of the Controlled Release Society. 2010; 144:324–331. [PubMed: 20219607]
73. Hak S, Helgesen E, Hektoen HH, Huuse EM, Jarzyna PA, Mulder WJ, Haraldseth O, Davies Cde L. The effect of nanoparticle polyethylene glycol surface density on ligand-directed tumor targeting studied in vivo by dual modality imaging. ACS nano. 2012; 6:5648–5658. [PubMed: 22671719]
74. Wang B, He X, Zhang Z, Zhao Y, Feng W. Metabolism of nanomaterials in vivo: blood circulation and organ clearance. Accounts of chemical research. 2013; 46:761–769. [PubMed: 23964655]
75. Kreuter J, Shamenkov D, Petrov V, Ramge P, Cychutek K, Koch-Brandt C, Alyautdin R. Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier. Journal of drug targeting. 2002; 10:317–325. [PubMed: 12164380]
76. Ogawara K, Furumoto K, Nagayama S, Minato K, Higaki K, Kai T, Kimura T. Pre-coating with serum albumin reduces receptor-mediated hepatic disposition of polystyrene nanosphere:

implications for rational design of nanoparticles. Journal of controlled release : official journal of the Controlled Release Society. 2004; 100:451–455. [PubMed: 15567509]

77. Hawkins MJ, Soon-Shiong P, Desai N. Protein nanoparticles as drug carriers in clinical medicine. Advanced drug delivery reviews. 2008; 60:876–885. [PubMed: 18423779]
78. Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Itty Ipe B, Bawendi MG, Frangioni JV. Renal clearance of quantum dots. Nature biotechnology. 2007; 25:1165–1170.
79. Cabral H, Matsumoto Y, Mizuno K, Chen Q, Murakami M, Kimura M, Terada Y, Kano MR, Miyazono K, Uesaka M, Nishiyama N, Kataoka K. Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. Nature nanotechnology. 2011; 6:815–823.
80. Baier G, Costa C, Zeller A, Baumann D, Sayer C, Araujo PH, Mailander V, Musyanovych A, Landfester K. BSA adsorption on differently charged polystyrene nanoparticles using isothermal titration calorimetry and the influence on cellular uptake. Macromolecular bioscience. 2011; 11:628–638. [PubMed: 21384550]
81. Smith PJ, Giroud M, Wiggins HL, Gower F, Thorley JA, Stolpe B, Mazzolini J, Dyson RJ, Rappoport JZ. Cellular entry of nanoparticles via serum sensitive clathrin-mediated endocytosis, and plasma membrane permeabilization. International journal of nanomedicine. 2012; 7:2045–2055. [PubMed: 22619541]
82. Patel PC, Giljohann DA, Daniel WL, Zheng D, Prigodich AE, Mirkin CA. Scavenger receptors mediate cellular uptake of polyvalent oligonucleotide-functionalized gold nanoparticles. Bioconjugate chemistry. 2010; 21:2250–2256. [PubMed: 21070003]
83. Lesniak A, Campbell A, Monopoli MP, Lynch I, Salvati A, Dawson KA. Serum heat inactivation affects protein corona composition and nanoparticle uptake. Biomaterials. 2010; 31:9511–9518. [PubMed: 21059466]
84. Chithrani BD, Ghazani AA, Chan WC. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano letters. 2006; 6:662–668. [PubMed: 16608261]
85. Dutta D, Sundaram SK, Teeguarden JG, Riley BJ, Fifield LS, Jacobs JM, Addleman SR, Kaysen GA, Moudgil BM, Weber TJ. Adsorbed proteins influence the biological activity and molecular targeting of nanomaterials. Toxicological sciences : an official journal of the Society of Toxicology. 2007; 100:303–315. [PubMed: 17709331]
86. Caracciolo G, Callipo L, De Sanctis SC, Cavaliere C, Pozzi D, Lagana A. Surface adsorption of protein corona controls the cell internalization mechanism of DC-Chol-DOPE/DNA lipoplexes in serum. Biochimica et biophysica acta. 2010; 1798:536–543. [PubMed: 19917267]
87. Paula AJ, Araujo Junior RT, Martinez DS, Paredes-Gamero EJ, Nader HB, Duran N, Justo GZ, Alves OL. Influence of protein corona on the transport of molecules into cells by mesoporous silica nanoparticles. ACS applied materials & interfaces. 2013; 5:8387–8393. [PubMed: 23841723]
88. Lundqvist M, Stigler J, Cedervall T, Berggard T, Flanagan MB, Lynch I, Elia G, Dawson K. The evolution of the protein corona around nanoparticles: a test study. ACS nano. 2011; 5:7503–7509. [PubMed: 21861491]
89. Caracciolo G, Cardarelli F, Pozzi D, Salomone F, Maccari G, Bardi G, Capriotti AL, Cavaliere C, Papi M, Lagana A. Selective Targeting Capability Acquired with a Protein Corona Adsorbed on the Surface of 1,2-Dioleoyl-3-trimethylammonium Propane/DNA Nanoparticles. ACS applied materials & interfaces. 2013
90. Zhang J, Landry MP, Barone PW, Kim JH, Lin S, Ulissi ZW, Lin D, Mu B, Boghossian AA, Hilmer AJ, Rwei A, Hinckley AC, Kruss S, Shandell MA, Nair N, Blake S, Sen F, Sen S, Croy RG, Li D, Yum K, Ahn JH, Jin H, Heller DA, Essigmann JM, Blankschtein D, Strano MS. Molecular recognition using corona phase complexes made of synthetic polymers adsorbed on carbon nanotubes. Nature nanotechnology. 2013

Highlights

- The nano-plasma interface consists of interaction between nanoparticles and biomolecules in the blood.
- The properties of nanoparticles and biomolecules change at the nano-plasma interface.
- Interactions with plasma components have implications for the toxicity, immunological recognition, targeting, biodistribution, intracellular uptake, and drug release of nanoparticles.
- The properties of the nano-plasma interface dictate the biocompatibility and efficacy of nanotherapeutics.
- The nano-plasma interface poses several challenges for nanomedicine, but it also presents opportunities for taking advantage of biomolecule-nanoparticle interactions.

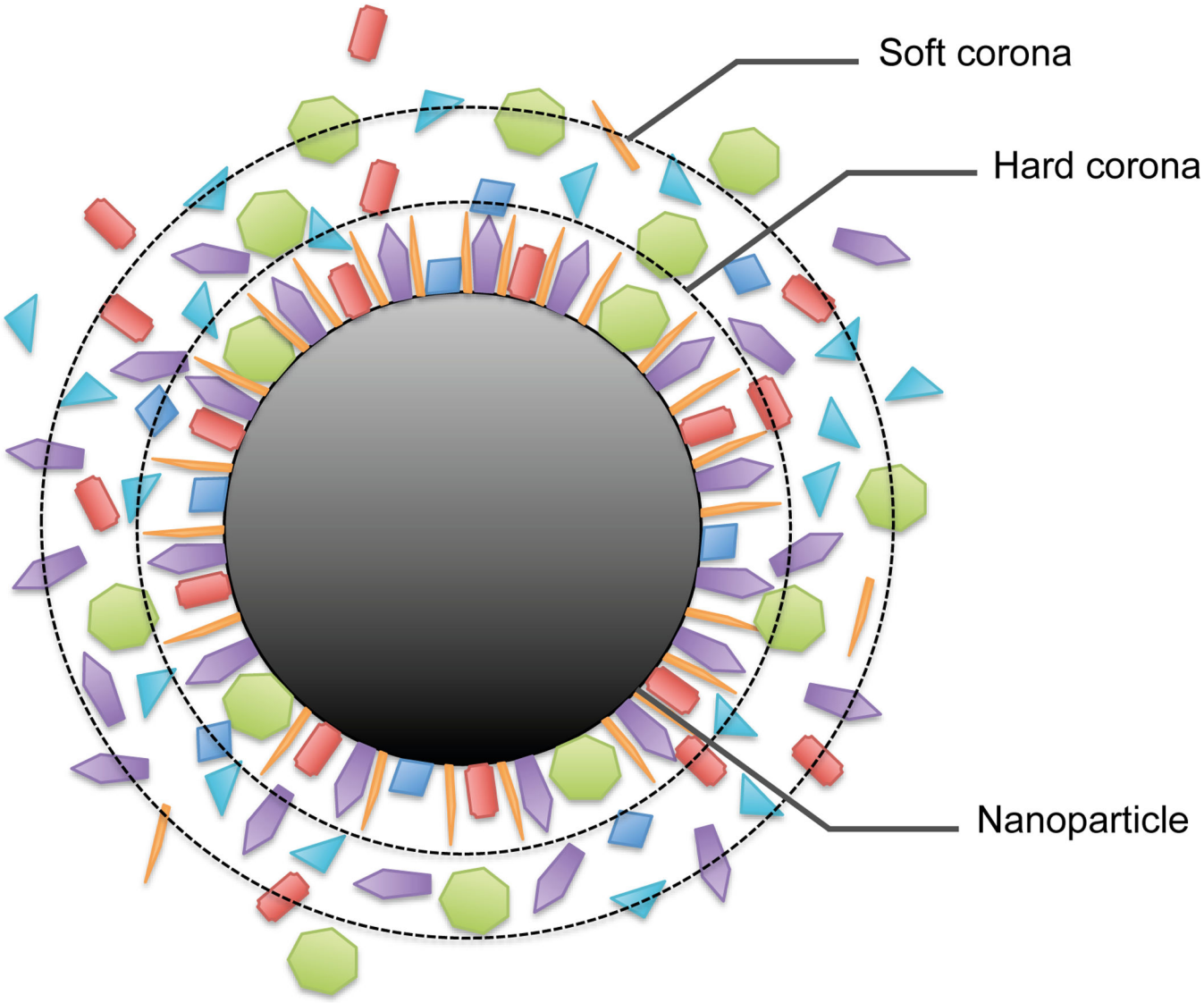

Figure 1.
Schematic representation of the current protein corona hypothesis. A hard and soft layer of proteins cover the surface of the nanoparticle. The proteins in the hard corona are more tightly associated with the particle surface, making them less dynamic than the proteins in the soft corona.

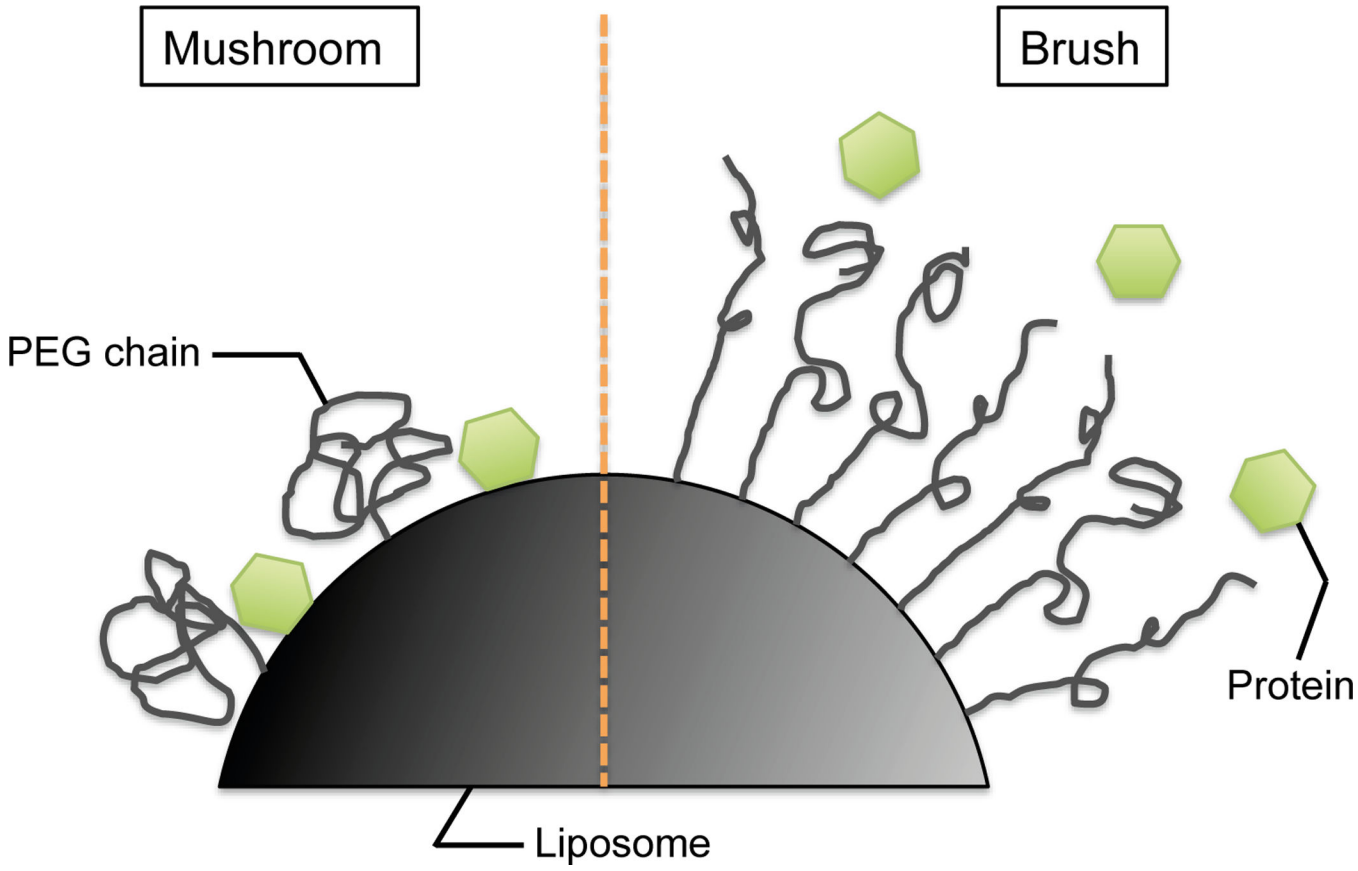

Figure 2.

Mushroom and brush conformation of polyethylene glycol (PEG) chains on the surface of a liposome. Low-density PEG chains form a mushroom structure, while high-density chains develop a brush conformation. In the mushroom conformation, proteins can more easily access the nanoparticle surface.

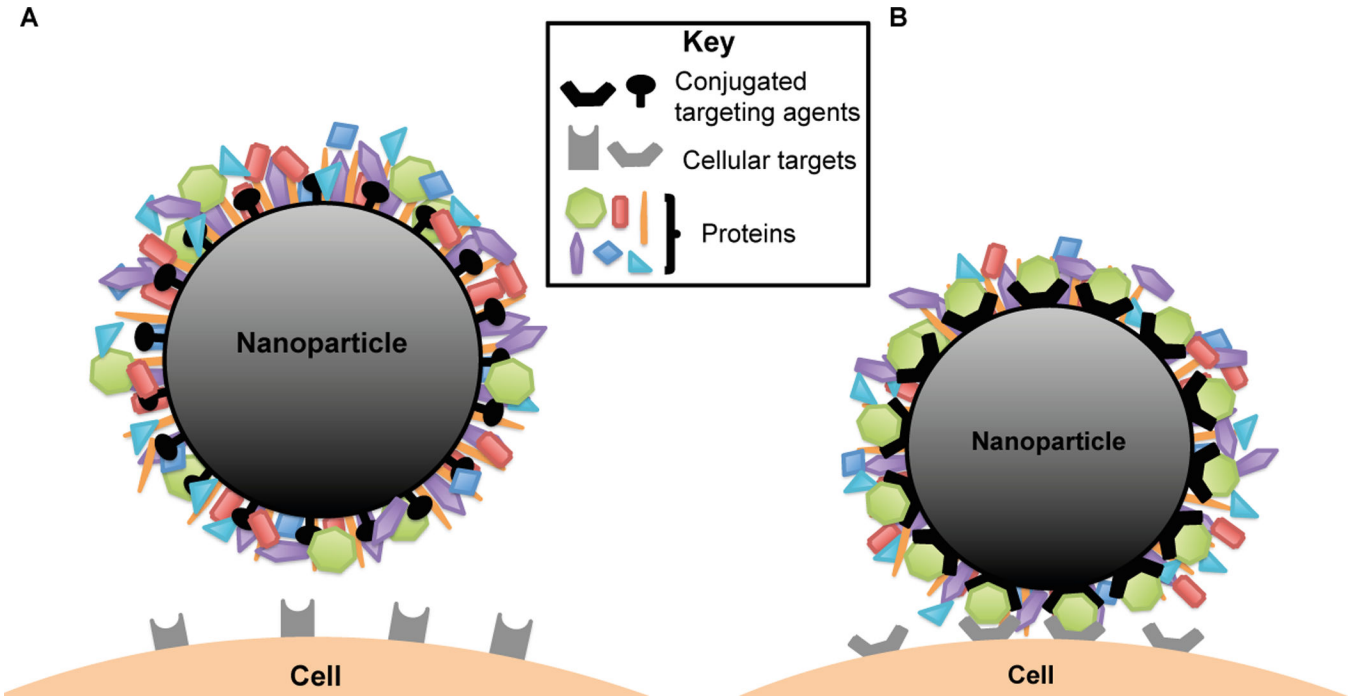

Figure 3.

Targeting approaches for nanoparticles. A) Conventional targeting strategy. Cell-specific targeting ligands on the nanoparticle surface are masked by a protein coating. The targeting moieties make contact with their respective targets after the protein corona has formed. B) Targeting of plasma proteins. Targeting ligands on the nanoparticle surface bind to plasma proteins, which also have cell-specific affinity. The targeting moieties make contact with their respective targets during corona formation, potentially making this strategy more effective.

Table 1

The nano-plasma interface

Components	Changes	Implications
Nanoparticle	• Size • Shape • Charge • Aggregation	• Toxicity • Immunological recognition • Molecular targeting • Biodistribution • Intracellular uptake • Drug release →Biocompatibility and efficacy
Proteins	• Structural modifications • Aggregation →Loss of functional activity	