Drug Delivery

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Controlling the Stealth Effect of Nanocarriers through Understanding the Protein Corona

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The past decade has seen a significant increase in interest in the use of polymeric nanocarriers in medical applications. In particular, when used as drug vectors in targeted delivery, nanocarriers could overcome many obstacles for drug therapy. Nevertheless, their application is still impeded by the complex composition of the blood proteins covering the particle surface, termed the protein corona. The protein corona complicates any prediction of cell interactions, biodistribution, and toxicity. In particular, the unspecific uptake of nanocarriers is a major obstacle in clinical studies. This Minireview provides an overview of what we currently know about the characteristics of the protein corona of nanocarriers, with a focus on surface functionalization that reduces unspecific uptake (the stealth effect). The ongoing improvement of nanocarriers to allow them to meet all the requirements necessary for successful application, including targeted delivery and stealth, are further discussed.

1. Introduction

The use of nanoscale materials for therapeutic purposes is probably one of the most exciting fields in nanotechnology. Engineered nanoparticles (NPs) are a promising tool and are currently under investigation for applications in drug delivery, diagnostics, imaging, and medical products such as implants. Owing to their small size of typically 10–100 nm,[1] they cross biological barriers and permeate into organs and tissues. Engineered nanoparticles can even enter cells through endocytotic mechanisms.[2] They have the potential for size-dependent interaction with cells, cell organelles, proteins, or DNA. Although the potential of NPs has been extensively studied, the topic of predicting nanoparticle cell interactions or biodistribution still needs to be examined.

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Nanotechnology enables materials to be specifically designed according to application requirements. Particular features of nanoparticles can be manipulated in order to gain unique physical, chemical, and biological properties and ultimately to design multifunctional nanoparticle platforms. Nanoparticles can be made from various materials, including inorganic materials such as gold, iron oxide, or silica; organic polymers such as polystyrene (PS), polylactic acid (PLA), or poly(lactic-co-glycolic acid) (PLGA); or biopolymers such as proteins, carbohydrates, or lipids.

Most applications in nanomedicine currently concentrate on the targeted delivery of drugs within the body.[3] Encapsulation of pharmaceuticals in nanoparticles or nanocapsules could potentially circumvent many problems and it has been proposed for use in cancer therapy. Chemotherapeutic agents like doxorubicin and paclitaxel were among the first agents to be delivered by nanocarriers with the potential to alter the biodistribution of drugs within the body. Owing to their cytotoxicity, chemotherapeutic drugs cause serious side effects, which can be minimized by using nanocarriers as targeted drug delivery systems. Localized treatment enabled by site-specific drug delivery could improve therapeutic efficiency. Furthermore, the encapsulated pharmaceuticals are protected from degradation and the solubility of water-insoluble drugs can be improved.

In addition to classical small-molecule substances, biopharmaceuticals such as proteins or nucleic acids are innovative drugs that could be used to treat a variety of medical conditions. They are extremely specific and potent but their high molecular mass and instability limit their administration.

The integration of biopharmaceuticals into nanosized carriers could overcome these limitations and decisively improve their pharmacokinetic characteristics on a macroscopic scale.[4] On a microscopic scale, entering the cell membrane is a major hurdle for these classes of biopharmaceuticals. Therefore, the endocytotic uptake of nanocarriers into cells is an important feature for circumventing this barrier. In order to be taken up through invagination of the cell membrane, the nanocarrier first needs to bind to the cell surface—a process inherently governed by the surface characteristics of the nanocarrier and in particular the protein corona attached to it. After attachment, the cell membrane wraps around the nanocarrier.

On the macroscopic scale, passive targeting of tumors is achieved as a consequence of the enhanced permeability and retention (EPR) effect, which has been exploited by several research groups.[5] The EPR effect is a consequence of the defective vasculature surrounding cancer tissue, which leads to enhanced uptake of the nanoparticles. Additionally, attachment of high-affinity ligands can further improve the targeting of tumors. Similarly, other cells such as cells of the immune system can be targeted, which can be exploited for immune-cell-based therapy. Conjugation to targeting moieties usually involves antibodies or ligands specific for particular cell receptors.

One of the targeting ligands employed is folic acid. This has been coupled to the surface of nanoparticles to target cancer cells overexpressing the folic acid receptor.[6] Another tumor marker overexpressed on cancer cell surfaces and used in experimental approaches as a target for directed therapy is the receptor EGFR-2 (HER2). Improved internalization has been reported for nanoparticles coated with anti-HER2 antibody and with folic acid for tumor targeting.[7] Anti-CD8 antibodies have been applied for the targeting of PLGA nanoparticles to CD8-expressing cells.[8] An effect of adsorbed proteins from the plasma on such targeting efforts has been pointed out,[9] but it seems that these problems can be overcome, as other groups have shown.[10]

To date, only very few nanocarrier drug systems are available on the market, including albumin nanoparticles containing paclitaxel (Abraxane, Celgene)[11] and doxorubicin liposomes (Doxil, Janssen),[12] both applied in cancer treatment. In contrast to the vast number of novel nanoscale systems produced, the low number of FDA-approved nanocarrier systems illustrates the complexity of their application and the hurdles of translating basic research into a pharmaceutically formulated drug.

Despite all of the advantages nanomedicine potentially offers, the application of nanocarriers is still restricted by insufficient knowledge of their interactions with the biological environment. Thorough assessment of biological responses evoked by nanocarriers is essential. One major challenge is the formation of a protein corona. Upon contact with physiological fluids, nanomaterials are immediately covered with proteins (Figure 1).[13–15] This rapidly forming protein corona dramatically alters the physicochemical properties of the nanocarriers, including hydrodynamic size, surface charge, and aggregation behavior. The original chemical identity of the surface of the bare nanoparticle is thus transformed into a biological identity. Although this has been known for quite

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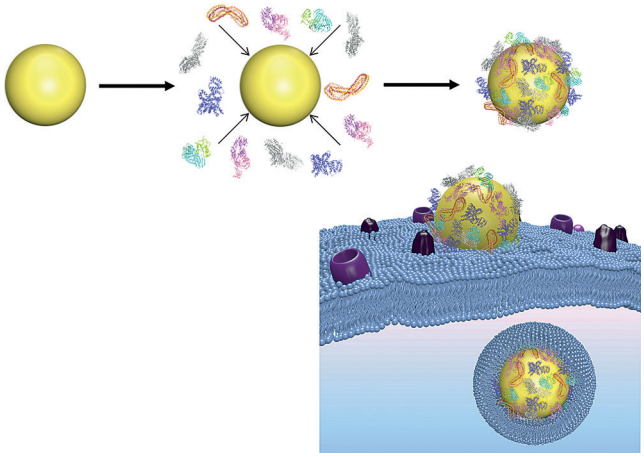

Figure 1. Formation of the protein corona on the nanocarrier surface can influence interaction with the cell membrane.

some time, it is only recently that such complex protein mixtures could be deciphered by means of liquid chromatography coupled to mass spectrometry (LC–MS). Furthermore, the biological effect on interaction with cell membranes and the influence on the mechanism of cellular uptake by the adsorbed proteins have now been pinpointed. Therefore, the corona defines the biological identity of nanoparticles, influencing cytotoxicity, body distribution, and endocytosis into specific cells.[16,17] When nanocarriers are introduced into the body, what the cells actually see is the protein corona.[13] Thus, prediction of nanocarrier–cell interactions is only possible if the protein corona is taken into account.

Studies analyzing the protein corona have established that there are two layers of proteins an inner layer of irreversibly attached proteins known as the hard corona, and an outer layer of loosely bound and continuously interchanging proteins forming the soft corona.[14,18] The allocation is defined by the affinity of the proteins for the nanocarrier surface.

It has been hypothesized that in the case of the hard corona, proteins interact directly with the nanocarrier surface, while proteins belonging to the soft corona interact with proteins that are already bound through weak protein–protein interactions.[16] Since the hard corona remains bound to the surface for a longer period of time and can survive processes such as endocytosis and translocation to different physiological environments, it is thought that the hard corona plays a more important role in biological responses than the soft corona.[16]

Protein corona formation has been generally observed and described for various nanoparticle materials, including polystyrene,[13,15,18,19] silica,[15,19,20] metal,[21,22] and lipids.[23] Two general consequences of protein adsorption for nanoparticles are: an increase in size and the creation of a negative surface charge owing to the anionic character of most blood proteins.[23]

2. Analytical Methods for Characterization

Given the importance of the effect of the protein corona on nanomaterials designed for medical applications, the development of analytical methods for its characterization is essential. Different techniques have been used to analyze the various parameters of the protein corona.

The binding affinities of single proteins or protein mixtures can be analyzed by fluorescence spectroscopy,[24,25] isothermal titration calorimetry (ITC),[26] or surface plasmon resonance (SPR).[27] Cedervall et al. were the first to utilize ITC and SPR for protein corona analysis.[14] ITC monitors the change in heat that results from protein adsorption onto nanomaterials and can provide information on protein binding affinities and stoichiometry.

With differential centrifugal sedimentation (DCS)[28] and dynamic light scattering (DLS),[13,29] the thickness of the protein corona can be determined. DLS makes use of the light scattering from particles in solution to determine the size-distribution profile. Changes in the hydrodynamic radius are detected by using this method, which provides information on increases in particle size and protein-induced NP aggregation. In DCS, particle-size distributions are measured by using a spinning disc with a defined viscous sucrose gradient. The primary information is the time taken by the particles to travel from the center of the disk through the gradient to a detector placed at the outer side.[19] DLS and DCS are among the few methods that are applicable in situ.[16]

Several techniques for monitoring conformational changes in proteins attaching to the NP surface are available. With circular dichroism (CD),[25,30] protein secondary structures can be determined, but the technique is limited to single proteins and cannot be applied in complex protein mixtures. Further techniques that can be used to document structural changes are Fourier transform infrared (FTIR)[25,31] or Raman spectroscopy.[32]

In order to obtain data on the effect of the protein corona on cellular uptake, aggregation, biodistribution, clearance, and toxicity, it is first necessary to provide protein identification. For this purpose, the hard corona needs to be purified after nanoparticle incubation in plasma or serum. Isolation of NP–protein complexes from the surrounding medium is a central challenge of investigating the protein corona since it may disrupt the interactions between particles and proteins.[14] Centrifugation is the most commonly used method for protein isolation since it exploits the size differences and density of nanoparticles relative to free proteins. For nanoparticles with magnetic properties, separation from the incubation medium by means of a strong magnet is possible and has been applied to protein corona purification.[33–35] Once the NP–protein complexes have been isolated and the adhering proteins removed from the NP surface, they are separated by polyacrylamide gel electrophoresis (PAGE) or chromatography. The identification is then realized by mass spectrometry (MS), which provides both qualitative and quantitative information. The identification rate of proteins attached to nanoparticles has increased enormously. While in first studies using 2D-PAGE, only 10 to 20 proteins could be detected,[34,36] nowadays up to 500 proteins can be identified.[15]

3. Composition of the Protein Corona

Knowledge of the composition of the protein corona is a prerequisite to understanding the physiological reactions triggered by nanocarriers in vivo. Nanocarriers designed for biomedical applications are often administered intravenously and are thus exposed to blood proteins. As a result, the composition of the protein corona is determined by over 3700 proteins present in plasma, with concentrations varying from $50\,mg\,mL^{-1}$ for albumin to $5\,pg\,mL^{-1}$ for interleukin 6.[37]

Proteins of the hard corona are more easily isolated because of their longer residence time and higher affinity and have thus been investigated in most studies dealing with the protein corona.[14,18,38] Several studies have shown that the composition of the protein corona does not merely reflect the abundance of particular proteins in blood, although highly abundant proteins have a much higher chance of interacting

as first proteins with the NP surface (the Vroman effect, see Section 4).[23,39]

Furthermore, it has been shown that there is no universal protein corona for all nanocarriers, but instead complex varying composites.[16] Independent of the bulk material and surface functionalities, highly abundant blood proteins such as albumin, immunoglobulin G (IgG), and fibrinogen are associated with a wide range of NPs.[14,21,39] Albumin, for example, was determined to be the main protein on gold nanoparticles[21] and was also detected on silica or polystyrene NPs[15,39] or liposomes.[40]

These highly abundant proteins often display higher rates of association and dissociation compared to, for example, apolipoproteins, which are frequently identified as strongly adsorbing proteins on the vast majority of investigated nanoparticles.[16,41,42] The high affinity of apolipoproteins for many nanoparticle types could be based on hydrophobic interactions and could additionally be driven by size-dependent interactions.[42] Together with phospholipids, apolipoproteins assemble lipoproteins such as high-density lipoprotein (HDL) or low-density lipoprotein (LDL), with sizes in the nanoscale range. These transport vehicles carry phospholipids and cholesterol through the bloodstream. Apolipoproteins that form HDL vesicles, such as APOA1, A2, A4, C1, C3, D, E or J, were found on the surface of various nanoparticles consisting of distinct materials.[15,18,38,39,43] Additionally, phospholipids have been identified in the corona of copolymer NPs, thus indicating that intact HDL particles might interact with the particle surface.[17,44]

A second group of proteins often identified on NPs are the complement factors.[39] As part of innate immunity, the complement system helps to remove foreign substances from the body. In response to a trigger, normally a pathogen, the complement cascade, which consists of over 30 proteins, is activated. Their main task is to tag the pathogen surface for identification by phagocytes. Complement C3 is a widely observed example in the protein corona of gold,[45] lipid-coated,[46] and polymeric nanoparticles.[47]

Besides the qualitative and quantitative composition of the protein corona, the orientation and availability of epitopes of the absorbed proteins have only recently been addressed.[48] By expanding on these results, a molecular picture of the protein corona can be revealed.

4. Evolution of the Protein Corona

As early as 1962, Leo Vroman postulated the theory of dynamic adsorption process of blood proteins on surfaces. While studying protein attachment on flat surfaces, he revealed high adsorption of fibrinogen at an intermediate incubation time and assumed subsequent replacement of fibrinogen by other proteins.[49] This phenomenon of sequential competitive adsorption, known as the Vroman effect, has been applied to other proteins and other surfaces.

For nanocarriers, it is assumed that highly abundant and motile blood proteins such as albumin, IgG, and fibrinogen adhere to the surface initially but are later displaced by proteins with lower abundance but higher affinity, such as apolipoproteins or coagulation factors.[16,50] This sequential binding pattern of plasma proteins has been confirmed for several polymeric model nanoparticles[38,50] and solid lipid NPs.[51] Additionally, an increase in the number of rather large proteins with molecular weights over 200 kDa with increasing incubation time has been reported.[23] Eventually, a point in time is reached at which the continuous exchange of proteins does not affect the protein corona composition any further and an equilibrium is established.[44] Rapid formation of the hard corona has been detected after just one minute,[23] and it is generally recognized that after one hour, a stable composition is achieved.[13]

Recently, an extensive study on polystyrene and silica nanoparticles revealed that protein fingerprints were already determined at the earliest exposure time of 0.5 min and did not significantly change qualitatively after prolonged plasma exposure.[15] Interestingly, in the case of iron oxide nanoparticles,[52] oil-in-water nanoemulsions,[53] and gold nanoparticles,[54] no competitive protein displacement over time was observed. This is in line with Vroman's suggestion that displacement takes place within seconds. Investigation of the protein corona during such a short incubation time is difficult. Accordingly, in most studies analyzing adsorption kinetics, 0.5 to 5 minutes is the shortest time range in use.[15,54] Other studies, especially those confirming the Vroman effect, use different dilutions of plasma instead to simulate the early stages of protein adsorption.[38,50,51]

Dynamic variation of the composition of the protein corona occurs not only over time but also during the journey of a nanocarrier through the body. Nanocarriers entering through the lung will be covered by proteins different from those present on the nanocarrier surface when it is administered intravenously; the composition evolves in accordance with the changing environment. This characteristic protein pattern could possibly be employed to trace the transport of nanoparticles throughout the body or even within a cell. Lundqvist et al. analyzed the corona composition on polystyrene nanoparticles transferred from plasma to cytosolic fluid.[55] They reported that while some proteins, such as apolipoprotein A1 (APOA1), are replaced by cytosolic proteins, other proteins of the plasma fingerprint are retained. Furthermore, the protein corona enables us to monitor intracellular trafficking pathways that are employed by nanoparticles.[35] This is highly significant since it adds to our knowledge of intracellular trafficking pathways through an unbiased method of analyzing the plethora of proteins involved in intracellular trafficking. Intracellular compartments hitherto not known to be involved in the shuttling of nanocarriers through the cell have been elucidated.[35,56]

The time dependency of protein adsorption is of special relevance because nanocarrier interactions with the environment in biomedical applications take place on different timescales. Protein attachment within the bloodstream may be a question of minutes, but interaction with cells of distant organs may be relevant hours or even days after exposure.[3]

5. Parameters that Affect the Protein Corona

Several studies have attempted to connect the physicochemical properties of the nanoparticle, such as material size, shape, charge, and chemical functionality, with the corona composition. Even surface roughness may play a role here.[57] However, owing to their easy tunability, a wide range of distinct NPs have been synthesized, which makes reliable predictions of nanoparticle–protein interactions even more difficult.

We are not able to draw universal conclusions regarding NP size. In the case of certain polymeric nanoparticles, variation in particle size resulted in a primarily quantitative influence on the protein composition.[38,39] On the other hand, a size-dependent qualitative influence on the protein pattern has also been reported for gold[58] and polystyrene nanoparticles.[18] In their study on polystyrene nanoparticles, Lundqvist and colleagues assigned both NP size and surface properties an important role in determining protein adsorption.[18] Many interactions contribute to protein attachment, such as van der Waals interactions, electrostatic interactions, hydrophobic interactions, and hydrogen bonding.[59] All these forces might be relevant in driving protein adsorption. Although factors such as electrostatic interactions between functionalized nanoparticles and charged side chains of proteins play a role in protein binding, it is now understood that adsorption is mainly driven by hydrophobic interactions (Figure 2).[39,60]

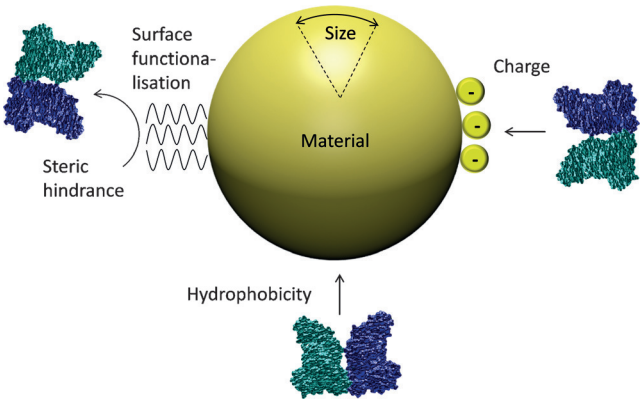

Figure 2. The characteristics of a nanocarrier that influence protein adsorption.

A correlation between NP hydrophobicity and protein association has been demonstrated by the enhanced adsorption of proteins on hydrophobic compared to hydrophilic surfaces.[3,61] Furthermore, it has been suggested that a negative surface charge on NPs correlates with reduced protein adsorption, since most serum proteins also have a negative charge.[62] However, despite their surface charge, anionic NPs are equally covered by proteins.[63] Modulating the surface charge of nanoparticles significantly influences the quality of the corona composition, that is, the protein pattern.[14,64] Preferential binding of negatively charged proteins to positively charged NPs and vice versa has been reported for some nanocarriers,[21,65] but it does not appear to be a general rule. By contrast, protein binding affinities have been shown to be independent of the NP charge and are not determined by the isoelectric point of the proteins.[35,39] Although the proteins in the corona cannot be predicted by their isoelectric point, the nanocarrier surface charge decisively determines the composition of the protein corona.

While the particle material can also influence protein adsorption, surface characteristics such as hydrophobicity and charge seem to play a more important role in determining the specific composition of the protein corona.[64]

Apart from the nanocarrier surface properties, the composition of the protein corona is highly dependent on the protein source. If the corona is determined after incubation in blood plasma, proteins of the coagulation system are often identified.[18,39,66] By contrast, serum is depleted of coagulation factors, especially fibrinogen. While it has been reported that variations exist in the protein pattern of the NP corona formed in fetal bovine serum (FBS) or human plasma,[67] the importance of the protein source for protein corona formation has recently been extensively examined with regard to nanoparticle uptake.[68] This study underlines the importance of a careful choice of the protein source, since it shows a significant difference in cell interactions for NPs incubated in FBS, human serum, and human plasma. It also points out that different anticoagulants have an influence on uptake. While polystyrene NPs with a protein corona formed in FBS are effectively taken up by HeLa cells and the macrophage-like cell line RAW264.7, human serum and human citrate plasma inhibited internalization. Most interestingly, NPs incubated with human heparin plasma were only internalized by RAW264.7, while no uptake was observed for HeLa cells. In this case, heparin was able to adsorb onto the protein corona as another component that was not detected in the standard proteomics workup.

Significantly, protein corona composition is affected by the protein source, and not only in terms of anticoagulant or species. Even plasma from different individuals results in different effects. Protein adsorption has been compared in human plasma samples obtained from patients with distinct diseases. A marked variation in the protein corona composition was observed, thus indicating the existence of personalized protein coronas.[69] Additionally, parameters such as concentration[70] or temperature[71] of the protein source are important factors in protein interactions.

6. Stealth Materials and Specific Proteins that Confer the Stealth Effect

As in the case of all foreign substances, nanocarriers are cleared from the bloodstream by cells of the mononuclear phagocyte system (MPS), also known as reticuloendothelial system (RES). This part of the immune system consists of phagocytes such as monocytes and macrophages, which are mainly located in liver, spleen, lungs, and lymph nodes. Phagocytosis of nanocarriers is promoted by opsonizing proteins, such as IgG and complement proteins, which label the nanoparticles as foreign material. The rapid removal of

circulating nanocarriers from the bloodstream is a major problem, since it prevents the nanocarriers from reaching their destination in the body. Hence, nanocarriers have been equipped with protein-repellent surfaces to reduce protein attachment and extend circulation time in the body. The resulting stealth nanocarriers are promising tools for avoiding activation of the immune system and allow successful drug delivery.

Owing to a higher degree of opsonization, hydrophobic nanoparticles have a shorter circulation half-life. Hence, nanoparticles have been coated with hydrophilic molecules to protect them from opsonization and recognition by cells of the MPS.[72] Covalent attachment of polyethylene glycol (PEG), also known as PEGylation, is the standard approach and has been used to increase the circulation time of a variety of polypeptides,[73] polymeric NPs,[74,75] and liposomes.[76]

Despite intensive research, the mechanism behind the protein resistance of PEGylated surfaces is not yet fully understood. It is assumed that the protein-repellent properties of PEG-coated surfaces are the result of steric repulsion caused by adsorbed water molecules through hydrogen bonding. The nature of the PEG derivatives used, including chain length, conformation, and surface density, plays an important role in the effectiveness of protein resistance.[77] Walkey et al. have demonstrated that PEG grafting leads to a decrease in total protein adsorption on gold nanoparticles, and this effect increases with increasing density of the PEG coating.[45] The amount of protein could also be reduced by increasing the molecular weight of the PEG grafted onto the gold nanoparticles from 2 to 20 kDa.[54] Different conformations of PEG are formed depending on the grafting density. A mushroom-like configuration is formed if the surface coverage of PEG is rather low, while densely packed PEG on the nanoparticle surface results in a brush-like structure. High steric protection has predominantly been shown for brush conformations and mushroom/brush intermediates.[78]

Although modifying nanocarriers with PEG reduces unspecific protein adsorption[74,79] and extends blood residence time,[80] it cannot completely prevent protein corona formation.[43,45,74] Despite the widespread use of PEG for medicinal applications, drawbacks have been recognized. For example, PEG is not biodegradable and may accumulate in the body. Additionally, the development of PEG antibodies[81] and severe hypersensitivity reactions have been reported.[82] Antibody recognition can lead to accelerated blood clearance with repeated systemic administration.[83] These factors necessitate the search for alternatives to PEG. Zwitterionic molecules such as polybetaines or polysaccharides can also generate hydrophilic shells when coupled to nanoparticles and have been discussed as PEG alternatives.[72] The biodegradability of polysaccharides makes them especially interesting for medical applications. Hydroxyethyl starch (HES) has been used to synthesize degradable polymeric nanocarriers.[84–86] As a starch derivative, HES can be enzymatically hydrolyzed by amylases in the body. Furthermore, protein-repellent characteristics have been postulated for HES and prevention of unspecific uptake of HES nanocapsules (HES-NCs) into HeLa cells has been demonstrated.[85,87] HES nanocapsules were also shown to exhibit prolonged circulation time in blood, which is necessary for the therapeutic efficacy of encapsulated drugs.[86]

In addition to the inhibition of unspecific cellular uptake, directed targeting to specific cells is important for the successful application of nanocarriers as drug delivery vehicles. In a recent study, PEGylated HES-NCs equipped with different targeting molecules to promote uptake into dendritic cells were analyzed regarding their protein adsorption.[10] An overall low protein adsorption with a distinct protein pattern and a minimal influence of the targeting moieties on the protein corona composition were observed. This is a promising indication for specific targeting under physiological conditions, since the targeting of specific cells is possible only if protein adsorption does not impair the selective binding of the targeting molecules coupled to the nanocarriers. Salvati et al. demonstrated the importance of this matter.[9] In their study, PEGylated silica nanoparticles functionalized with transferrin lost their targeting specificity for the transferrin receptor, expressed on cancer cells (A549 cells), upon exposure to serum. In contrast, the protein corona formed around the HES nanocapsules did not impair recognition by the corresponding receptors. Cellular uptake of the NCs by dendritic cells proved that the targeting moieties are active and accessible to the biological receptors after incubation with human plasma.[10]

Another innovative polymer class that has been proposed as an alternative to PEG is poly(phosphoester)s (PPEs).[88] Their high degree of modularity and biodegradability makes them attractive for nanocarrier systems. Functionalization with poly(ethylene ethyl phosphate) (PEEP) reduces protein adsorption to PS-NPs and uptake into RAW264.7 macrophages in a similar way to that of PEG. Interestingly, the stealth effect provoked by PEG and PEEP was shown to be dependent on protein adsorption, and one specific protein, clusterin (or APOJ), was found to play a crucial role in this mechanism. These findings give us a new view on the mechanism of the stealth effect, in which adsorption of specific proteins onto the nanocarriers confers the stealth effect. As a result, the underlying surface functionalization needs to attract the desired proteins instead of being responsible for this effect by itself (Figure 3, for further proteins that inhibit unspecific cellular uptake, see below. This contradicts the view that the lack of protein alone is responsible for the stealth effect.

7. Effect of the Protein Corona on Physiological Responses

The interaction of nanocarriers with cells, including immunological responses and internalization pathways, is dominated by adsorbed proteins. Even intracellular trafficking and localization are dependent on the adsorbed proteins.[89] These effects can be disadvantageous as described above for the unspecific phagocytosis of opsonized NPs. Two further aspects of protein adsorption have raised concerns. Firstly, adsorption of proteins can trigger conformational changes in the protein structure.[90] Structural changes can give rise to functionality loss,[91] and exposure of cryptic

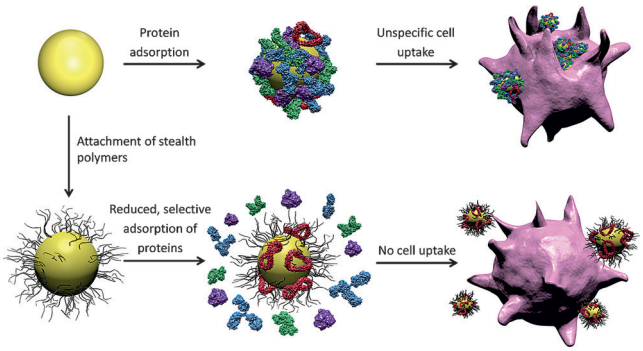

Figure 3. New perspectives on the stealth effect. Unfunctionalized nanocarriers are opsonized and recognized by phagocytic cells. Attachment of stealth polymers leads to reduced protein adsorption, but specific proteins such as clusterin adsorb to the functionalized nanocarriers and prevent uptake by immune cells.

epitopes could trigger an inappropriate immune response.[92] Secondly, as discussed in Section 6, proteins adhering to nanocarriers equipped with antibodies or ligands can impair targeting by masking the ligand and thus provoking a loss of recognition by its specific cell receptor.[9,93]

On the other hand, protein adsorption can also be beneficial. A general reduction in unspecific cellular uptake caused by the presence of a protein corona has been described for several NPs,[94] which additionally results in lower toxicity.[95] The prevention of random internalization is probably triggered by reduced interaction of the nanoparticles with the cell membrane resulting from a shielding effect from the adsorbed proteins. Proteins that induce this effect against phagocytic cells are called dysopsonins. Albumin and apolipoproteins have been described as proteins with dysopsonizing character, which prolongs the blood half-life of the nanocarriers.[19,23,64] In fact, coating of polymeric nanoparticles with human serum albumin (HSA) has been shown to increase their blood circulation time.[96]

Apolipoproteins A4 and C3 have also been identified to reduce NP uptake into human mesenchymal stem cells, which supports the general role of lipoproteins as dysopsonins.[97] Interestingly, APOH (or beta2 glycoprotein I) has been described as a protein that promotes the internalization of nanoparticles into hMSCs, which leads to a more complex view of the effect of apolipoproteins in the protein corona.[97,98]

Originally, studies for stealth materials were determined by the search for protein-repellent surface modifications and the need to increase blood circulation times. However, the perception of the protein corona is now changing. Since protein corona formation is inevitable and even the stealth effect seems to be dependent on the adsorption of proteins,[88] the concept of its exploitation for targeted delivery is emerging. In contrast to unspecific interactions between proteins and cells mentioned above, specific uptake of nanocarriers could be promoted if receptor-specific proteins were to reside in the protein corona.[95]

Several blood proteins have known cellular binding sites, for example, the transferrin receptor, which is present on a variety of cells.[99] Endothelial cells express an albumin-binding glycoprotein,[100] and multiple receptors for apolipoprotein complexes have been described.[101] The best-known receptor for lipoproteins is the scavenger receptor class B type 1 (SR-B1), which is expressed not only in the brain, liver, and intestines, but also on endothelial cells and macrophages.[23] It is responsible for bidirectional lipid transfer between LDL, HDL, and cells.[102] Nanoparticles covered with apolipoproteins could thus mimic HDL and interact with scavenger receptors. A prostate carcinoma cell line expressing high levels of scavenger receptors has already been successfully targeted with lipid NPs with a protein corona rich in apolipoproteins.[23]

The coating of nanocarriers with single proteins has been exploited for the targeting of specific organs. For example, apolipoproteins APOE, APOA1, and APOB-100 promote transport into the central nervous system.[103] APOE attached to the surface of nanoparticles is thought to facilitate the transport of NPs across the blood–brain barrier, probably through interaction with lipoprotein receptors in the membranes of brain capillary endothelial cells.[104] Apart from opportunities for developing ground breaking innovative neurotherapies through this approach, these findings are also very important when considering neurotoxicity.[42] Apolipoproteins could also be used to selectively target hepatocytes in the liver.[105] Additionally, fetuin has been shown to be involved in NP uptake into hepatic macrophages via scavenger receptors.[106]

So far, the biological impact has only been defined in the case of a few corona proteins. Understanding exactly which proteins determine the fate of nanocarriers is crucial to ensuring the success of designed targeted nanocarriers. Optimizing the properties of nanocarriers in order to exploit the protein corona for specific biomedical applications is of high value and interest.[107]

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[1] M. E. Davis, Z. Chen, D. M. Shin, *Nat. Rev. Drug Discovery* **2008**, *7*, 771–782.
[2] J. Dausend, A. Musyanovych, M. Dass, P. Walther, H. Schrezenmeier, K. Landfester, V. Mailaender, *Macromol. Biosci.* **2008**, *8*, 1135–1143; A. Musyanovych, J. Dausend, M. Dass, P. Walther, V. Mailaender, K. Landfester, *Acta Biomater.* **2011**, *7*, 4160–4168.
[3] M. Rahman, S. Laurent, N. Tawil, L. Yahia, M. Mahmoudi, *Protein-Nanoparticle-Interactions*, *The Bio-Nano Interface*, Springer Series in Biophysics, *Vol. 15*, Springer-Verlag Berlin, **2013**.
[4] S. Mitragotri, P. A. Burke, R. Langer, *Nat. Rev. Drug Discovery* **2014**, *13*, 655–672.
[5] J. M. Morachis, E. A. Mahmoud, A. Almutairi, *Pharmacol. Rev.* **2012**, *64*, 505–519.

[6] Z. Zhang, J. Jia, Y. Lai, Y. Ma, J. Weng, L. Sun, *Bioorg. Med. Chem.* **2010**, *18*, 5528 – 5534.

[7] L. E. van Vlerken, M. M. Amiji, *Expert Opin. Drug Delivery* **2006**, *3*, 205 – 216.

[8] A. Bicho, I. N. Peca, A. C. Roque, M. M. Cardoso, *Int. J. Pharm.* **2010**, *399*, 80 – 86.

[9] A. Salvati, A. S. Pitek, M. P. Monopoli, K. Prapainop, F. B. Bombelli, D. R. Hristov, P. M. Kelly, C. Aberg, E. Mahon, K. A. Dawson, *Nat. Nanotechnol.* **2013**, *8*, 137 – 143.

[10] B. Kang, P. Okwieka, S. Schöttler, S. Winzen, J. Langhanki, K. Mohr, T. Opatz, V. Mailander, K. Landfester, F. R. Wurm, *Angew. Chem. Int. Ed.* **2015**, *54*, 7436 – 7440; *Angew. Chem.* **2015**, *127*, 7544 – 7548.

[11] D. D. Von Hoff, T. Ervin, F. P. Arena, E. G. Chiorean, J. Infante, M. Moore, T. Seay, S. A. Tjulandin, W. W. Ma, M. N. Saleh, M. Harris, M. Reni, S. Dowden, D. Laheru, N. Bahary, R. K. Ramanathan, J. Tabernero, M. Hidalgo, D. Goldstein, E. Van Cutsem, X. Wei, J. Iglesias, M. F. Renschler, *N. Engl. J. Med.* **2013**, *369*, 1691 – 1703.

[12] Y. Barenholz, *J. Controlled Release* **2012**, *160*, 117 – 134.

[13] D. Walczyk, F. B. Bombelli, M. P. Monopoli, I. Lynch, K. A. Dawson, *J. Am. Chem. Soc.* **2010**, *132*, 5761 – 5768.

[14] T. Cedervall, I. Lynch, S. Lindman, T. Berggard, E. Thulin, H. Nilsson, K. A. Dawson, S. Linse, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 2050 – 2055.

[15] S. Tenzer, D. Docter, J. Kuharev, A. Musyanovych, V. Fetz, R. Hecht, F. Schlenk, D. Fischer, K. Kiouptsi, C. Reinhardt, K. Landfester, H. Schild, M. Maskos, S. K. Knauer, R. H. Stauber, *Nat. Nanotechnol.* **2013**, *8*, 772 – 781.

[16] C. D. Walkey, W. C. Chan, *Chem. Soc. Rev.* **2012**, *41*, 2780 – 2799.

[17] E. Hellstrand, I. Lynch, A. Andersson, T. Drakenberg, B. Dahlback, K. A. Dawson, S. Linse, T. Cedervall, *FEBS J.* **2009**, *276*, 3372 – 3381.

[18] M. Lundqvist, J. Stigler, G. Elia, I. Lynch, T. Cedervall, K. A. Dawson, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 14265 – 14270.

[19] M. P. Monopoli, D. Walczyk, A. Campbell, G. Elia, I. Lynch, F. B. Bombelli, K. A. Dawson, *J. Am. Chem. Soc.* **2011**, *133*, 2525 – 2534.

[20] N. P. Mortensen, G. B. Hurst, W. Wang, C. M. Foster, P. D. Nallathamby, S. T. Retterer, *Nanoscale* **2013**, *5*, 6372 – 6380.

[21] E. Casals, T. Pfaller, A. Duschl, G. J. Oostingh, V. Puntes, *ACS Nano* **2010**, *4*, 3623 – 3632.

[22] Z. J. Deng, G. Mortimer, T. Schiller, A. Musumeci, D. Martin, R. F. Minchin, *Nanotechnology* **2009**, *20*, 455101.

[23] A. L. Barrán-Berdón, D. Pozzi, G. Caracciolo, A. L. Capriotti, G. Caruso, C. Cavaliere, A. Riccioli, S. Palchetti, A. Laganà, *Langmuir* **2013**, *29*, 6485 – 6494.

[24] C. A. Royer, *Chem. Rev.* **2006**, *106*, 1769 – 1784; C. Rocker, M. Potzl, F. Zhang, W. J. Parak, G. U. Nienhaus, *Nat. Nanotechnol.* **2009**, *4*, 577 – 580.

[25] L. Shang, Y. Wang, J. Jiang, S. Dong, *Langmuir* **2007**, *23*, 2714 – 2721.

[26] G. Baier, C. Costa, A. Zeller, D. Baumann, C. Sayer, P. H. H. Araujo, V. Mailänder, A. Musyanovych, K. Landfester, *Macromol. Biosci.* **2011**, *11*, 628 – 638; S. Winzen, S. Schoettler, G. Baier, C. Rosenauer, V. Mailaender, K. Landfester, K. Mohr, *Nanoscale* **2015**, *7*, 2992 – 3001.

[27] Y. Cheng, M. Wang, G. Borghs, H. Chen, *Langmuir* **2011**, *27*, 7884 – 7891; S. Linse, C. Cabaleiro-Lago, W.-F. Xue, I. Lynch, S. Lindman, E. Thulin, S. E. Radford, K. A. Dawson, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 8691 – 8696.

[28] A. S. Pitek, D. O'Connell, E. Mahon, M. P. Monopoli, F. B. Bombelli, K. A. Dawson, *PLoS One* **2012**, *7*, e40685.

[29] I. Delfino, S. Cannistraro, *Biophys. Chem.* **2009**, *139*, 1 – 7; J. Bhattacharya, U. Choudhuri, O. Siwach, P. Sen, A. K. Dasgupta, *Nanomed. Nanotechnol. Biol. Med.* **2006**, *2*, 191 – 199.

[30] Z. J. Deng, M. Liang, M. Monteiro, I. Toth, R. F. Minchin, *Nat. Nanotechnol.* **2011**, *6*, 39 – 44; S. H. D. P. Lacerda, J. J. Park, C. Meuse, D. Pristinski, M. L. Becker, A. Karim, J. F. Douglas, *ACS Nano* **2010**, *4*, 365 – 379.

[31] K. K. Chittur, *Biomaterials* **1998**, *19*, 357 – 369; T. Wang, J. Bai, X. Jiang, G. U. Nienhaus, *ACS Nano* **2012**, *6*, 1251 – 1259.

[32] M. Shao, L. Lu, H. Wang, S. Luo, D. Ma, *Microchim. Acta* **2009**, *164*, 157 – 160; X. C. Shen, X. Y. Liou, L. P. Ye, H. Liang, Z. Y. Wang, *J. Colloid Interface Sci.* **2007**, *311*, 400 – 406; L.-J. Xu, C. Zong, X.-S. Zheng, P. Hu, J.-M. Feng, B. Ren, *Anal. Chem.* **2014**, *86*, 2238 – 2245.

[33] M. Mahmoudi, M. A. Shokrgozar, S. Sardari, M. K. Moghadam, H. Vali, S. Laurent, P. Stroeve, *Nanoscale* **2011**, *3*, 1127 – 1138; M. Mahmoudi, S. Sant, B. Wang, S. Laurent, T. Sen, *Adv. Drug Delivery Rev.* **2011**, *63*, 24 – 46.

[34] K. Thode, M. Luck, W. Semmler, R. H. Muller, M. Kresse, *Pharm. Res.* **1997**, *14*, 905 – 910.

[35] U. Sakulkhu, M. Mahmoudi, L. Maurizi, J. Salaklang, H. Hofmann, *Sci. Rep.* **2014**, *4*, 5020.

[36] M. Lück, B. R. Paulke, W. Schröder, T. Blunk, R. H. Müller, *J. Biomed. Mater. Res.* **1998**, *39*, 478 – 485.

[37] B. Muthusamy, G. Hanumanthu, S. Suresh, B. Rekha, D. Srinivas, L. Karthick, B. M. Vrushabendra, S. Sharma, G. Mishra, P. Chatterjee, K. S. Mangala, H. N. Shivashankar, K. N. Chandrika, N. Deshpande, M. Suresh, N. Kannabiran, V. Niranjan, A. Nalli, T. S. K. Prasad, K. S. Arun, R. Reddy, S. Chandran, T. Jadhav, D. Julie, M. Mahesh, S. L. John, K. Palvankar, D. Sudhir, P. Bala, N. S. Rashmi, G. Vishnupriya, K. Dhar, S. Reshma, R. Chaerkady, T. K. B. Gandhi, H. C. Harsha, S. S. Mohan, K. S. Deshpande, M. Sarker, A. Pandey, *Proteomics* **2005**, *5*, 3531 – 3536; N. L. Anderson, N. G. Anderson, *Mol. Cell. Proteomics* **2002**, *1*, 845 – 867.

[38] T. Cedervall, I. Lynch, M. Foy, T. Berggad, S. C. Donnelly, G. Cagney, S. Linse, K. A. Dawson, *Angew. Chem. Int. Ed.* **2007**, *46*, 5754 – 5756; *Angew. Chem.* **2007**, *119*, 5856 – 5858.

[39] S. Tenzer, D. Docter, S. Rosfa, A. Wlodarski, J. Kuharev, A. Rekik, S. K. Knauer, C. Bantz, T. Nawroth, C. Bier, J. Sirirattanapan, W. Mann, L. Treuel, R. Zellner, M. Maskos, H. Schild, R. H. Stauber, *ACS Nano* **2011**, *5*, 7155 – 7167.

[40] J. E. Diederichs, *Electrophoresis* **1996**, *17*, 607 – 611.

[41] M. P. Monopoli, C. Aberg, A. Salvati, K. A. Dawson, *Nat. Nanotechnol.* **2012**, *7*, 779 – 786.

[42] I. Lynch, K. A. Dawson, *Nano Today* **2008**, *3*, 40 – 47.

[43] H. R. Kim, K. Andrieux, C. Delomenie, H. Chacun, M. Appel, D. Desmaele, F. Taran, D. Georgin, P. Couvreur, M. Taverna, *Electrophoresis* **2007**, *28*, 2252 – 2261.

[44] D. Dell'Orco, M. Lundqvist, C. Oslakovic, T. Cedervall, S. Linse, *PLoS One* **2010**, *5*, e10949.

[45] C. D. Walkey, J. B. Olsen, H. Guo, A. Emili, W. C. Chan, *J. Am. Chem. Soc.* **2012**, *134*, 2139 – 2147.

[46] S. Nagayama, K. Ogawara, Y. Fukuoka, K. Higaki, T. Kimura, *Int. J. Pharm.* **2007**, *342*, 215 – 221.

[47] E. AlléAemann, P. Gravel, J. C. Leroux, L. Balant, R. Gurny, *J. Biomed. Mater. Res.* **1997**, *37*, 229 – 234.

[48] P. M. Kelly, C. Aberg, E. Polo, A. O'Connell, J. Cookman, J. Fallon, Z. Krpetic, K. A. Dawson, *Nat. Nanotechnol.* **2015**, *10*, 472 – 479.

[49] L. Vroman, *Nature* **1962**, *196*, 476 – 477; L. Vroman, A. L. Adams, G. C. Fischer, P. C. Munoz, *Blood* **1980**, *55*, 156 – 159.

[50] T. Blunk, M. Luck, A. Calvor, D. F. Hochstrasser, J. C. Sanchez, B. W. Muller, R. H. Muller, *Eur. J. Pharm. Biopharm.* **1996**, *42*, 262 – 268.

[51] T. M. Göppert, R. H. Müller, *Int. J. Pharm.* **2005**, *302*, 172 – 186.

[52] M. Jansch, P. Stumpf, C. Graf, E. Rühl, R. H. Müller, *Int. J. Pharm.* **2012**, *428*, 125 – 133.

[53] S. Harnisch, R. H. Müller, *Eur. J. Pharm. Biopharm.* **2000**, *49*, 41 – 46.

[54] M. A. Dobrovolskaia, B. W. Neun, S. Man, X. Ye, M. Hansen, A. K. Patri, R. M. Crist, S. E. McNeil, *Nanomed. Nanotechnol. Biol. Med.* **2014**, *10*, 1453 – 1463.
[55] M. Lundqvist, J. Stigler, T. Cedervall, T. Berggard, M. B. Flanagan, I. Lynch, G. Elia, K. Dawson, *ACS Nano* **2011**, *5*, 7503 – 7509.
[56] L. Nuhn, S. Tomcin, K. Miyata, V. Mailaender, K. Landfester, K. Kataoka, R. Zentel, *Biomacromolecules* **2014**, *15*, 4111 – 4121; S. Tomcin, G. Baier, K. Landfester, V. Mailaender, *Int. J. Nanomed.* **2014**, *9*, 5471 – 5489.
[57] M. Mahmoudi, V. Serpooshan, *J. Phys. Chem. C* **2011**, *115*, 18275 – 18283; A. Schrade, V. Mailaender, S. Ritz, K. Landfester, U. Ziener, *Macromol. Biosci.* **2012**, *12*, 1459 – 1471.
[58] M. Schäffler, M. Semmler-Behnke, H. Sarioglu, S. Takenaka, A. Wenk, C. Schleh, S. M. Hauck, B. D. Johnston, W. G. Kreyling, *Nanotechnology* **2013**, *24*, 265103.
[59] A. A. Saie, M. Ray, M. Mahmoudi, V. M. Rotello, *Cancer Treat. Res.* **2015**, *166*, 245 – 273; S.-T. Yang, Y. Liu, Y.-W. Wang, A. Cao, *Small* **2013**, *9*, 1635 – 1653.
[60] M. S. Ehrenberg, A. E. Friedman, J. N. Finkelstein, G. Oberdorster, J. L. McGrath, *Biomaterials* **2009**, *30*, 603 – 610.
[61] A. Gessner, R. Waicz, A. Lieske, B. Paulke, K. Mader, R. H. Muller, *Int. J. Pharm.* **2000**, *196*, 245 – 249.
[62] J. Wolfram, Y. Yang, J. Shen, A. Moten, C. Chen, H. Shen, M. Ferrari, Y. Zhao, *Colloids Surf. B* **2014**, *124*, 17 – 24; F. Alexis, E. Pridgen, L. K. Molnar, O. C. Farokhzad, *Mol. Pharmaceutics* **2008**, *5*, 505 – 515.
[63] M. Mahmoudi, I. Lynch, M. R. Ejtehadi, M. P. Monopoli, F. B. Bombelli, S. Laurent, *Chem. Rev.* **2011**, *111*, 5610 – 5637; C. C. Fleischer, C. K. Payne, *J. Phys. Chem. B* **2012**, *116*, 8901 – 8907.
[64] P. Aggarwal, J. B. Hall, C. B. McLeland, M. A. Dobrovolskaia, S. E. McNeil, *Adv. Drug Delivery Rev.* **2009**, *61*, 428 – 437.
[65] A. Gessner, A. Lieske, B.-R. Paulke, R. H. Müller, *J. Biomed. Mater. Res. Part A* **2003**, *65A*, 319 – 326.
[66] M. Gasser, B. Rothen-Rutishauser, H. Krug, P. Gehr, M. Nelle, B. Yan, P. Wick, *J. Nanobiotechnol.* **2010**, *8*, 31.
[67] S. Laurent, C. Burtea, C. Thirifays, F. Rezaee, M. Mahmoudi, *J. Colloid Interface Sci.* **2013**, *392*, 431 – 445.
[68] S. Schöttler, K. Klein, K. Landfester, V. Mailänder, *Nanoscale* **2016**, *8*, 5526 – 5536.
[69] M. J. Hajipour, S. Laurent, A. Aghaie, F. Rezaee, M. Mahmoudi, *Biomater. Sci.* **2014**, *2*, 1210 – 1221.
[70] M. Ghavami, S. Saffar, B. A. Emamy, A. Peirovi, M. A. Shokrgozar, V. Serpooshan, M. Mahmoudi, *RSC Adv.* **2013**, *3*, 1119 – 1126.
[71] M. Mahmoudi, A. M. Abdelmonem, S. Behzadi, J. H. Clement, S. Dutz, M. R. Ejtehadi, R. Hartmann, K. Kantner, U. Linne, P. Maffre, S. Metzler, M. K. Moghadam, C. Pfeiffer, M. Rezaei, P. Ruiz-Lozano, V. Serpooshan, M. A. Shokrgozar, G. U. Nienhaus, W. J. Parak, *ACS Nano* **2013**, *7*, 6555 – 6562.
[72] Z. Amoozgar, Y. Yeo, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2012**, *4*, 219 – 233.
[73] A. Abuchowski, J. R. McCoy, N. C. Palczuk, T. van Es, F. F. Davis, *J. Biol. Chem.* **1977**, *252*, 3582 – 3586; K. R. Reddy, T. L. Wright, P. J. Pockros, M. Shiffman, G. Everson, R. Reindollar, M. W. Fried, P. P. Purdum, D. Jensen, C. Smith, W. M. Lee, T. D. Boyer, A. Lin, S. Pedder, J. DePamphilis, *Hepatology* **2001**, *33*, 433 – 438.
[74] R. Gref, M. Luck, P. Quellec, M. Marchand, E. Dellacherie, S. Harnisch, T. Blunk, R. H. Muller, *Colloids Surf. B* **2000**, *18*, 301 – 313.
[75] S. Essa, J. M. Rabanel, P. Hildgen, *Int. J. Pharm.* **2011**, *411*, 178 – 187; P. Ebrahimnejad, R. Dinarvand, M. R. Jafari, S. A. Tabasi, F. Atyabi, *Int. J. Pharm.* **2011**, *406*, 122 – 127.
[76] A. L. Klibanov, K. Maruyama, V. P. Torchilin, L. Huang, *FEBS Lett.* **1990**, *268*, 235 – 237; T. M. Allen, C. B. Hansen, D. E. L. de Menezes, *Adv. Drug Delivery Rev.* **1995**, *16*, 267 – 284.
[77] S. I. Jeon, J. H. Lee, J. D. Andrade, P. G. De Gennes, *J. Colloid Interface Sci.* **1991**, *142*, 149 – 158.
[78] A. Vonarbourg, C. Passirani, P. Saulnier, J. P. Benoit, *Biomaterials* **2006**, *27*, 4356 – 4373.
[79] K. Bergström, K. Holmberg, A. Safranj, A. S. Hoffman, M. J. Edgell, A. Kozlowski, B. A. Hovanes, J. M. Harris, *J. Biomed. Mater. Res.* **1992**, *26*, 779 – 790.
[80] T. Niidome, M. Yamagata, Y. Okamoto, Y. Akiyama, H. Takahashi, T. Kawano, Y. Katayama, Y. Niidome, *J. Controlled Release* **2006**, *114*, 343 – 347.
[81] T. Ishida, M. Ichihara, X. Wang, K. Yamamoto, J. Kimura, E. Majima, H. Kiwada, *J. Controlled Release* **2006**, *112*, 15 – 25; T. Ishida, H. Kiwada, *Biol. Pharm. Bull.* **2013**, *36*, 889 – 891.
[82] S. Shah, T. Prematta, N. F. Adkinson, F. T. Ishmael, *J. Clin. Pharmacol.* **2013**, *53*, 352 – 355.
[83] N. Bertrand, J. C. Leroux, *J. Controlled Release* **2012**, *161*, 152 – 163.
[84] M. Fichter, G. Baier, M. Dedters, L. Pretsch, A. Pietrzak-Nguyen, K. Landfester, S. Gehring, *Nanomed. Nanotechnol. Biol. Med.* **2013**, *9*, 1223 – 1234; H. Freichels, M. Wagner, P. Okwieka, R. G. Meyer, V. Mailander, K. Landfester, A. Musyanovych, *J. Mater. Chem. B* **2013**, *1*, 4338 – 4348.
[85] G. Baier, D. Baumann, J. M. Siebert, A. Musyanovych, V. Mailander, K. Landfester, *Biomacromolecules* **2012**, *13*, 2704 – 2715.
[86] B. Kang, P. Okwieka, S. Schöttler, O. Seifert, R. E. Kontermann, K. Pfizenmaier, A. Musyanovych, R. Meyer, M. Diken, U. Sahin, V. Mailänder, F. R. Wurm, K. Landfester, *Biomaterials* **2015**, *49*, 125 – 134.
[87] A. Besheer, J. Vogel, D. Glanz, J. Kressler, T. Groth, K. Mäder, *Mol. Pharmaceutics* **2009**, *6*, 407 – 415.
[88] S. Schöttler, G. Becker, S. Winzen, T. Steinbach, K. Mohr, K. Landfester, V. Mailander, F. R. Wurm, *Nat. Nanotechnol.* **2016**, *11*, 372 – 377.
[89] D. Hofmann, S. Tenzer, M. B. Bannwarth, C. Messerschmidt, S.-F. Glaser, H. Schild, K. Landfester, V. Mailänder, *ACS Nano* **2014**, *8*, 10077 – 10088; F. Bertoli, G. L. Davies, M. P. Monopoli, M. Moloney, Y. K. Gun′ko, A. Salvati, K. A. Dawson, *Small* **2014**, *10*, 3307 – 3315.
[90] N. Brandes, P. B. Welzel, C. Werner, L. W. Kroh, *J. Colloid Interface Sci.* **2006**, *299*, 56 – 69; L. Treuel, G. Nienhaus, *Biophys. Rev.* **2012**, *4*, 137 – 147.
[91] A. A. Vertegel, R. W. Siegel, J. S. Dordick, *Langmuir* **2004**, *20*, 6800 – 6807.
[92] I. Lynch, K. A. Dawson, S. Linse, *Sci. STKE* **2006**, pe14; A. E. Nel, L. Madler, D. Velegol, T. Xia, E. M. V. Hoek, P. Somasundaran, F. Klaessig, V. Castranova, M. Thompson, *Nat. Mater.* **2009**, *8*, 543 – 557.
[93] V. Mirshafiee, M. Mahmoudi, K. Lou, J. Cheng, M. L. Kraft, *Chem. Commun.* **2013**, *49*, 2557 – 2559.
[94] G. Wang, M. R. Papasani, P. Cheguru, P. J. Hrdlicka, R. A. Hill, *Nanomedicine* **2012**, *8*, 822 – 832; P. C. Patel, D. A. Giljohann, W. L. Daniel, D. Zheng, A. E. Prigodich, C. A. Mirkin, *Bioconjugate Chem.* **2010**, *21*, 2250 – 2256; X. Jiang, S. Weise, M. Hafner, C. Rocker, F. Zhang, W. J. Parak, G. U. Nienhaus, *J. R. Soc. Interface* **2010**, *7*, S5 – S13; A. Lesniak, F. Fenaroli, M. P. Monopoli, C. Aberg, K. A. Dawson, A. Salvati, *ACS Nano* **2012**, *6*, 5845 – 5857; A. Lesniak, A. Salvati, M. J. Santos-Martinez, M. W. Radomski, K. A. Dawson, C. Aberg, *J. Am. Chem. Soc.* **2013**, *135*, 1438 – 1444; O. Lunov, T. Syrovets, C. Loos, J. Beil, M. Delacher, K. Tron, G. U. Nienhaus, A. Musyanovych, V. Mailander, K. Landfester, T. Simmet, *ACS Nano* **2011**, *5*, 1657 – 1669.
[95] E. Brun, C. Sicard-Roselli, *Cancer Nanotechnol.* **2014**, *5*, 7.
[96] Q. Peng, S. Zhang, Q. Yang, T. Zhang, X. Q. Wei, L. Jiang, C. L. Zhang, Q. M. Chen, Z. R. Zhang, Y. F. Lin, *Biomaterials* **2013**, *34*, 8521 – 8530.

[97] S. Ritz, S. Schöttler, N. Kotman, G. Baier, A. Musyanovych, J. Kuharev, K. Landfester, H. Schild, O. Jahn, S. Tenzer, V. Mailänder, *Biomacromolecules* **2015**, *16*, 1311 – 1321.
[98] I. Brüstle, T. Simmet, G. U. Nienhaus, K. Landfester, V. Mailänder, *Beilstein J. Nanotechnol.* **2015**, *6*, 383 – 395.
[99] P. Ponka, C. N. Lok, *Int. J. Biochem. Cell Biol.* **1999**, *31*, 1111 – 1137.
[100] J. E. Schnitzer, *Am. J. Physiol.* **1992**, *262*, H246 – H254.
[101] H. R. Kim, S. Gil, K. Andrieux, V. Nicolas, M. Appel, H. Chacun, D. Desmaele, F. Taran, D. Georgin, P. Couvreur, *Cell. Mol. Life Sci.* **2007**, *64*, 356 – 364.
[102] V. I. Zannis, A. Chroni, M. Krieger, *J. Mol. Med.* **2006**, *84*, 276 – 294.
[103] H. R. Kim, K. Andrieux, S. Gil, M. Taverna, H. Chacun, D. Desmaele, F. Taran, D. Georgin, P. Couvreur, *Biomacromolecules* **2007**, *8*, 793 – 799; J. Kreuter, *Adv. Drug Delivery Rev.* **2012**, *64*, 213 – 222; J. Kreuter, T. Hekmatara, S. Dreis, T. Vogel, S. Gelperina, K. Langer, *J. Controlled Release* **2007**, *118*, 54 – 58.
[104] K. Michaelis, M. M. Hoffmann, S. Dreis, E. Herbert, R. N. Alyautdin, M. Michaelis, J. Kreuter, K. Langer, *J. Pharmacol. Exp. Ther.* **2006**, *317*, 1246 – 1253; A. Zensi, D. Begley, C. Pontikis, C. Legros, L. Mihoreanu, S. Wagner, C. Büchel, H. von Briesen, J. Kreuter, *J. Controlled Release* **2009**, *137*, 78 – 86.
[105] C. L. Bisgaier, M. V. Siebenkas, K. J. Williams, *J. Biol. Chem.* **1989**, *264*, 862 – 866; P. C. Rensen, M. C. van Dijk, E. C. Havenaar, M. K. Bijsterbosch, J. K. Kruijt, T. J. van Berkel, *Nat. Med.* **1995**, *1*, 221 – 225.
[106] S. Nagayama, K. i. Ogawara, K. Minato, Y. Fukuoka, Y. Takakura, M. Hashida, K. Higaki, T. Kimura, *Int. J. Pharm.* **2007**, *329*, 192 – 198.
[107] A. Cifuentes-Rius, H. de Puig, J. C. Y. Kah, S. Borros, K. Hamad-Schifferli, *ACS Nano* **2013**, *7*, 10066 – 10074; J. C. Kah, J. Chen, A. Zubieta, K. Hamad-Schifferli, *ACS Nano* **2012**, *6*, 6730 – 6740; C. D. Walkey, J. B. Olsen, F. Y. Song, R. Liu, H. B. Guo, D. W. H. Olsen, Y. Cohen, A. Emili, W. C. W. Chan, *ACS Nano* **2014**, *8*, 2439 – 2455.

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