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Nanoparticle Surface Charge Mediates the Cellular Receptors Used by Protein–Nanoparticle Complexes

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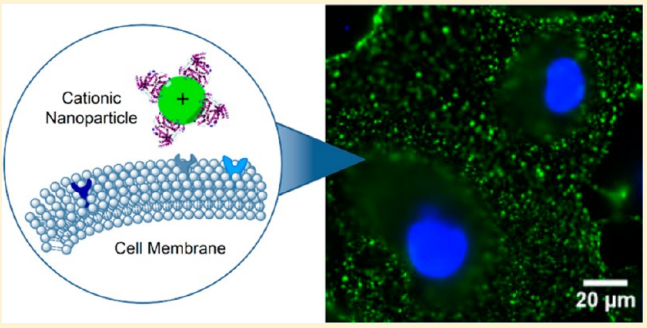
Supporting Information

ABSTRACT: Nanoparticles are increasingly important for biological applications ranging from drug delivery to cellular imaging. In the course of these applications, nanoparticles are exposed to a complex environment of extracellular proteins that can be adsorbed onto the surface of the nanoparticle, altering nanoparticle–cell interactions. We have investigated how proteins found in blood serum affect the binding of nanoparticles to the surface of cells. Using fluorescence microscopy, we find that the cellular binding of cationic nanoparticles is enhanced by the presence of serum proteins, while the binding of anionic nanoparticles is inhibited. We have determined that this difference in cellular binding is due to the use of distinct cellular receptors. Competition assays, quantified with flow cytometry, show that the protein–nanoparticle complex formed from the cationic nanoparticles binds to scavenger receptors on the cell surface. Interestingly, the protein–nanoparticle complex formed from anionic nanoparticles binds to native protein receptors. As nanoparticles become increasingly important for *in vivo* applications, we expect these results will inform the design of nanoparticles with improved cellular binding.



INTRODUCTION

Nanoparticles (NPs) have important biological applications ranging from drug and gene delivery to the fluorescent labeling of single cells.[1−7] Most, if not all, of these applications require the NP to come into contact with a complex milieu of extracellular proteins. For example, NPs exposed to blood will encounter blood serum proteins, a diverse mixture of hundreds of distinct proteins.[8−10] These proteins rapidly adsorb onto the surface of NPs.[11−13] The molecular picture of this protein layer, or protein "corona", on the surface of NPs has been characterized in the literature.[11,14−17] After exposure to serum proteins, a short-lived corona dominated by albumin, the most abundant serum protein,[8] is initially present. Over time, this "soft" corona can be replaced by a "hard" corona composed of less abundant, higher affinity proteins such as apolipoproteins.

Most important has been the insight that the proteins adsorbed onto the NP surface, rather than the NP, mediate the interaction of the cell with the NP. Serum proteins adsorb onto NPs in solution and remain bound to the NP during cellular binding and internalization.[18,19] Many serum proteins have dedicated cellular binding sites.[20−22] This suggests that serum proteins can influence the cellular binding of NPs by both the formation of a protein–NP complex and by competing with the NP for receptors on the plasma membrane. Serum proteins dominate the interaction of the NP with the immune system and alter cellular internalization pathways.[23] This previous work suggests that NPs in biological environments are more realistically described as a protein–NP complex in which the protein can alter interactions with the cell membrane and may be partially responsible for NP internalization and transport through the cell.

Of specific interest has been the role of the surface charge of the NP in the adsorption of serum proteins on the NP surface. A general picture has emerged suggesting that neutral or negatively charged NPs help to shield NPs from adsorption of the negatively charged serum proteins but that these NPs have only a low affinity for the negatively charged binding sites on the plasma membrane.[24] In comparison, positively charged NPs risk adsorption of anionic serum proteins but readily bind and are internalized by anionic proteoglycans on the cell surface.[25] The physical reality is much more complex, as anionic polystyrene,[11,23,26] citrate-modified gold NPs,[27] and poly(acrylic acid)-modified gold nanorods have all been shown to form complexes with serum proteins.[28] Surface plasmon resonance studies of gold surfaces show that albumin, a highly abundant serum protein,[8−10] binds more readily to anionic carboxylate-modified surfaces than cationic amine-modified surfaces, likely due to the presence of multiple lysine residues.[29]

Our goal was to determine how the cellular binding of cationic and anionic NPs is affected by extracellular serum proteins. To address this question, we carried out a side-by-side comparison of the cellular binding of NPs in the presence of

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serum proteins using NPs of the same composition, with opposite charges, in the same cell line. We found that both cationic and anionic NPs formed protein−NP complexes when exposed to serum proteins. While the presence of serum proteins enhanced the cellular binding of cationic NPs, it inhibited the binding of anionic NPs. We determined that this difference in cellular binding is due to the cellular receptors used by the protein−NP complexes. Protein−NP complexes formed from cationic NPs bind to scavenger receptors on the cell surface. In comparison, the protein−NP complexes formed from anionic NPs compete with serum proteins in solution for native protein receptors. We expect these results will be useful for the many applications in which NPs are exposed to extracellular proteins.

EXPERIMENTAL METHODS

Nanoparticles (NPs). Fluorescent polystyrene NPs (FluoSpheres, Invitrogen) were used in all cellular binding experiments. The diameter provided by the supplier is used to denote the NP: 87 nm amine-modified (C29029), 200 nm amine-modified (F8764), 40 nm carboxylate-modified (F8795), and 200 nm carboxylate-modified (F8811) NPs. Experimental values are provided in Table 1.

Table 1. TEM Diameter (d_{TEM}), Hydrodynamic Diameter (d_h), and Zeta Potential (ZP) of the NPs Used in the Course of Experiments

NPs	d_{TEM} (nm)	d_h (nm)	ZP (mV)
87 nm NH_2	87 ± 6	152.7 ± 3.2	39.2 ± 3.6
200 nm NH_2	200 ± 11	270.1 ± 9.3	19.9 ± 3.4
40 nm COOH	45 ± 6	60.04 ± 0.91	−43.3 ± 1.5
200 nm COOH	210 ± 10	236.2 ± 3.3	−31.0 ± 1.6

Dynamic Light Scattering (DLS) and Zeta Potential Measurements. The hydrodynamic diameter and zeta potentials of the NPs were measured with a Malvern Zetasizer (Nano-ZS, Malvern Instruments) in disposable cuvettes. Solutions of NPs in water were measured at the following concentrations: 87 nm amine-modified (173 pM); 200 nm amine-modified (15 pM); 200 nm carboxylate-modified (13 pM); and 40 nm carboxylate-modified (3.3 nM). Measurements were carried out in triplicate. Hydrodynamic diameter and zeta potential data was acquired from ≥12 runs per measurement and 30 runs per measurement, respectively. Zeta potential measurements were run in general purpose mode, and the Smoluchowski approximation was used to convert the electrophoretic mobility to a zeta potential.

Cell Culture. African green monkey kidney epithelial cells (BS-C-1, ATCC) were maintained in a 37 °C, 5% carbon dioxide environment in Minimum Essential Medium (MEM, Invitrogen, 61100061) with 10% (v/v) fetal bovine serum (FBS, Invitrogen, 10437028). Cells were passaged every 3 days. For fluorescence imaging, cells were cultured in 35 mm glass-bottom cell culture dishes (MatTek). Nuclei were stained with 27 μM 4′,6-diamidino-2-phenylindole dilactate (DAPI, Invitrogen, D3571) at 37 °C for 1 h in MEM supplemented with 10% FBS.

Fluorescence Microscopy. The cellular binding of NPs was imaged with an epifluorescence microscope (Olympus IX7) using a 1.20 N.A., 60x, water immersion objective (Olympus). Emission was detected with an EMCCD (DU-897, Andor). All images for comparison were acquired with the same exposure time and gain. Image J (http://rsb.info.nih.gov/ij/) was used for analysis. Brightness and contrast were set equally for all images for comparison.

Gel Electrophoresis. NPs incubated in MEM supplemented with 10% FBS were washed by repeated centrifugation (16,000*g* for 10 min) and resuspension in water. The supernatant was collected after each wash. After the final wash, the sample was suspended in buffer containing 6% SDS (New England Biolabs, #B7703S) to remove the protein from the NP surface. Supernatant was diluted by 50% in Laemmli buffer (Boston Bioproducts, BP-110R), boiled for 5 min, and then loaded onto the gel. The supernatant from the first wash was diluted by an additional factor of 10 in water to avoid overloading the gel. The undiluted supernatant is shown in Figure S1 in the Supporting Information. Proteins were separated on a 4−20% gradient mini-protean gel (Bio-Rad, 456-1094) at 40 mA and 130 V along with a 5−225 kDa molecular weight marker (Lonza, 50547). Proteins were stained with Simply Blue Safe Stain (Invitrogen, LC6060) for 1 h.

Competition Assay. Binding competition studies used polyinosinic acid (Sigma-Aldrich, P4154) as a competitor for scavenger receptors and polyadenylic acid (Sigma-Aldrich, P9403) as a control. The competitor or control was incubated with the cells for 20 min in MEM supplemented with 10% FBS prior to the addition of amine-modified NPs or MEM alone prior to the addition of carboxylate-modified NPs. Cells were incubated with NPs at 4 °C in the presence of competitor for 10 min. Cells were rinsed twice with PBS with calcium and magnesium (Invitrogen, 14040182) and twice with PBS without calcium and magnesium (Invitrogen, 14190250). To remove the cells from the MatTek dishes and put them into suspension for flow cytometry (BD LSR II, BD Biosciences), the cells were incubated in a 10 mM solution of ethylenediaminetetraacetic acid (EDTA, Mallinckrodt, 4931-04) in PBS without calcium or magnesium for 30 min at 37 °C. Cells were rinsed twice with Leibovitz's L-15 buffer (Invitrogen, 21083027) by centrifugation at 10,000*g* for 8 min or 5,000*g* for 5 min (200 nm carboxylate-modified NPs) and kept on ice for at least 1 h prior to flow cytometry. NP fluorescence was excited with a 488 nm excitation source and collected with a 530/30 nm bandpass filter. Between 5,000 and 14,000 cells were used to calculate the mean fluorescence (n = 3−5). Histograms were analyzed using Weasel 3.0.1 (Walter and Eliza Hall Institute of Medical Research, Victoria, Australia).

RESULTS AND DISCUSSION

In the course of experiments, we used cationic, amine-modified polystyrene NPs and anionic, carboxylate-modified polystyrene NPs (Fluospheres, Invitrogen). Within our lab, the hydrodynamic diameter (d_h) and zeta potential (ZP) of the NPs was measured using a Nano-ZS Zetasizer (Table 1). NPs were diluted in water, and measurements were carried out in triplicate. The NP diameter measured by TEM (d_{TEM}), supplied by Invitrogen, is also provided in Table 1. Throughout the text, we use the diameter provided in the catalog to refer to the NP. For example, carboxylate-modified NPs with a d_{TEM} of 45 nm and a d_h of 60 nm are described as 40 nm NPs.

Cationic and Anionic NPs Form Protein−NP Complexes. We first determined whether both cationic and anionic NPs would form protein−NP complexes when exposed to extracellular serum proteins. Cells are typically cultured in an aqueous solution of amino acids, vitamins, inorganic salts, and glucose, described as the "medium", supplemented with serum.

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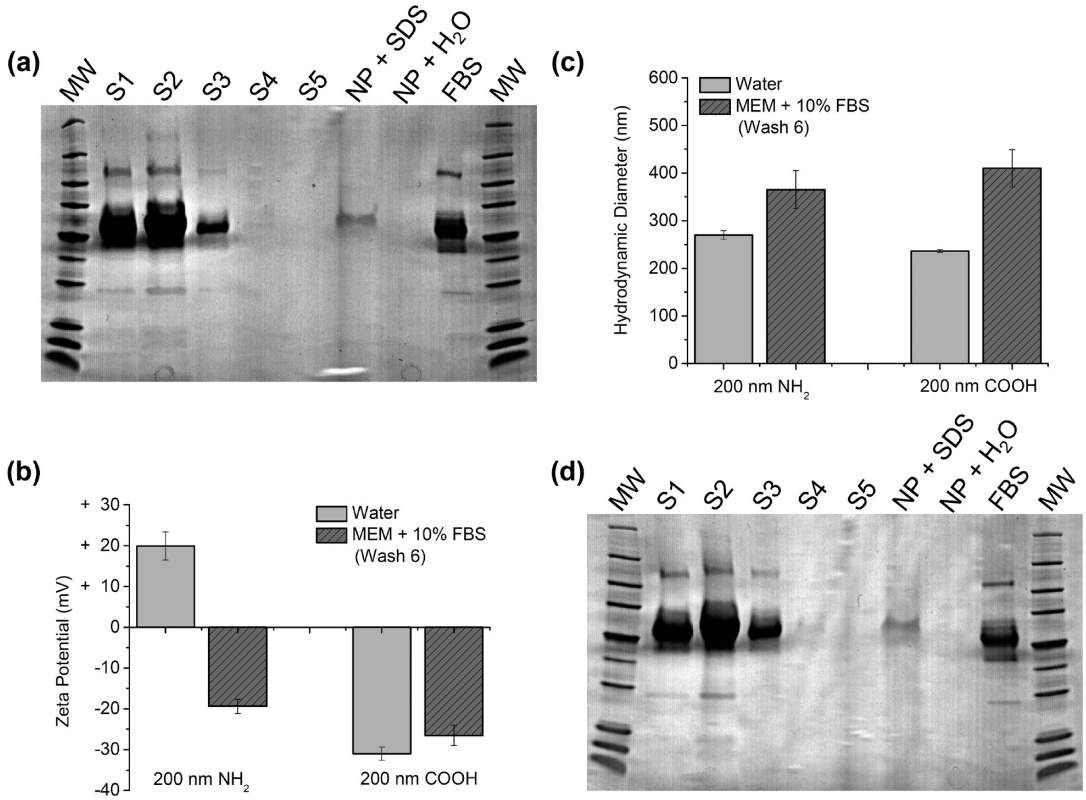

Figure 1. Formation of protein−NP complexes confirmed with gel electrophoresis, zeta potential, and hydrodynamic diameter measurements. (a) SDS-PAGE of supernatants (S) following repeated centrifugation and wash steps of the 200 nm amine-modified NPs. S1 was diluted by a factor of 10 to avoid overloading the gel (Figure S1, Supporting Information). After five washes (S5), protein is no longer visible in the supernatant. SDS removes the corona of protein adsorbed on the NP (NP + SDS) following the washes. In the absence of SDS, protein is not removed from the NP (NP + H_2O). FBS, in the absence of NPs, is used for comparison. MW marker shows 225, 150, 100, 75, 50, 35, 25, 15, 10, and 5 kDa. (b) Zeta potential of the NPs incubated with MEM supplemented with 10% FBS after six washes. (c) Hydrodynamic diameter after six washes. (d) SDS-PAGE of the 200 nm carboxylate-modified NPs using the same approach as described for part a.

Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) is commonly used for cell culture, and we used this mixture as a representative biological environment. A combination of gel electrophoresis, zeta potential, and hydrodynamic diameter measurements were used to characterize NPs after incubation in MEM supplemented with 10% FBS. The cationic NPs are expected to bind to the anionic serum proteins, as has been observed previously with zeta potential measurements and fluorescently labeled serum proteins.[18,19] This was confirmed by incubating the 200 nm amine-modified NPs (15 pM) with MEM supplemented with 10% FBS for 10 min at 4 °C, identical to the conditions used for cellular assays. The solution of NPs and serum proteins was then centrifuged (16,000*g*, 10 min) and resuspended in water six times. After each wash, the supernatant was collected, run on an polyacrylamide gel, and stained with Simply Blue Safe Stain for 1 h. After five washes, no protein was detected in the supernatant, demonstrating that unbound protein had been removed from the NP−protein mixture. The NPs were then resuspended in a solution of 6% SDS, a detergent that is expected to remove any proteins that remain bound to the NP. Following resuspension in SDS, a protein band at ∼66 kDa is visible in the gel (Figures 1a and S1 (Supporting Information), NP + SDS). In the absence of SDS, no protein is visible (NP + H_2O). This demonstrates that incubation in MEM supplemented with 10% FBS results in the adsorption of proteins on the 200 nm cationic NPs. The protein−NP complex that remained following the final wash, in the absence of SDS, was used for zeta potential and hydrodynamic diameter measurements (Figure 1b and c). These measurements show that the protein−NP complex formed with the cationic NP becomes anionic (−19.4 ± 1.8 mV) and increases in diameter (365.1 ± 39.8 nm). Similar results were obtained for the 87 nm amine-modified NPs, although these NPs were more difficult to pellet, resulting in greater conductivity and polydispersity (Figure S2, Supporting Information).

We next determined whether the 200 nm anionic NPs formed a protein−NP complex, similar to the cationic NPs, or if they were resistant to protein adsorption and remained bare NPs in the presence of FBS. The adsorption of serum proteins on the NP was investigated using gel electrophoresis, zeta potential, and hydrodynamic diameter measurements as described for the cationic NPs. Like the cationic NPs, protein was detected on "washed" anionic NPs following gel electrophoresis and SDS treatment (Figure 1d, NP + SDS). The charge of the anionic NP increased to −26.5 ± 2.5 mV (Figure 1b), more similar to the zeta potential of BSA (−22 mV, data not shown), and the diameter of the NP increased (410.2 ± 38.8 nm, Figure 1c). This demonstrates that exposure to FBS results in the adsorption of proteins on the 200 nm anionic NPs, in good agreement with previous results showing the adsorption of serum proteins on 100 nm carboxylate-modified polystyrene NPs.[23] The 40 nm carboxylate-modified NPs were too small to pellet and could not be analyzed after washing.

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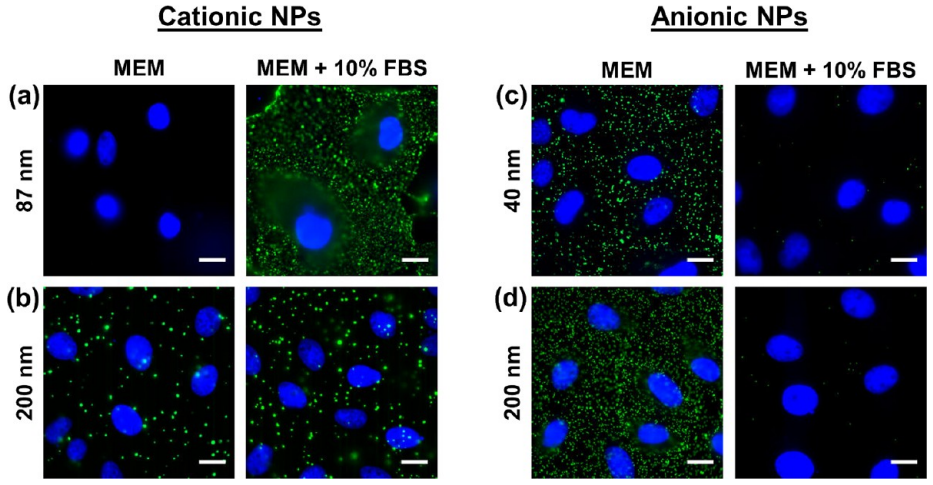

Figure 2. Fluorescence microscopy images of NPs (green) bound to BS-C-1 cells at 4 °C in MEM and MEM supplemented with 10% FBS. Nuclei are stained with DAPI (blue). At higher NP concentrations, cellular binding is independent of FBS (Figure S3, Supporting Information). Cationic NPs are amine-modified, and anionic NPs are carboxylate-modified. Scale bar is 20 μm.

Using three different methods, we show that serum proteins remain bound to both cationic and anionic NPs following multiple wash steps that remove free and weakly bound proteins. On the basis of the molecular weight of the protein removed in the SDS wash (Figure 1a and d), it is likely that BSA is the main protein adsorbed on the NPs, although lower abundance proteins would not be detected using this approach. This is an interesting result, as it shows both cationic and anionic NPs form a protein−NP complex. The protein−NP complex formed for both cationic and anionic NPs appears to be similar: both types of NPs are net anionic following exposure to 10% FBS, and both form a corona of mainly BSA.

Cellular Binding of Cationic NPs is Enhanced by Serum Proteins. To compare the cellular binding of the protein−NP complexes formed from the cationic and anionic NPs, we examined the cellular binding of the NPs in the presence and absence of excess serum proteins. We first compared the cellular binding of NPs in MEM, which lacks proteins, to MEM supplemented with 10% FBS. Cells were cooled to 4 °C for 20 min before the addition of NPs and were maintained at 4 °C during 10 min of incubation with the NPs. At 4 °C, NP internalization is blocked, allowing us to image cellular binding independently of internalization. After 10 min at 4 °C, cells were rinsed twice with phosphate buffered saline (PBS) and then imaged using epifluorescence microscopy. The concentration of NPs incubated with the cells is provided. We find that the 87 nm cationic NPs (34 pM) have minimal binding to cells in the absence of FBS and significant binding in the presence of FBS (Figure 2a). A similar increase in binding was observed for 200 nm cationic NPs (15 pM) in the presence of FBS (Figure 2b). We expect that the lower zeta potential of the 200 nm NPs results in greater direct interaction with the plasma membrane in the absence of FBS. These results show that the formation of a protein−NP complex enhances the cellular binding of cationic NPs. They suggest that the protein adsorbed on the NP is responsible for cellular binding and that this protein−NP complex does not compete with free proteins in solution for cellular binding sites.

Cellular Binding of Anionic NPs Is Inhibited by Serum Proteins. Unlike the cationic NPs, the cellular binding of both the 40 nm (26 pM) and 200 nm (13 pM) anionic NPs was inhibited by the addition of serum proteins (Figure 2c and d). As the anionic NPs form a protein−NP complex when exposed to FBS (Figure 1), it is the cellular binding of this protein−NP complex that is inhibited by the excess serum proteins in solution. This suggests that the protein−NP complex formed from the anionic NPs competes with the free proteins in solution for cellular binding sites. Our results are in good agreement with recent experiments that showed the cellular uptake of 49 and 100 nm anionic polystyrene NPs is inhibited by the presence of serum proteins.[30] On the basis of our results, which distinguish cellular binding from uptake, it is likely that decreased binding of anionic NPs in the presence of serum proteins led to the observed decrease in uptake. In addition, the results for polystyrene NPs are in good agreement with previous work showing that the cellular uptake of gold NPs functionalized with DNA or siRNA is inhibited by the presence of serum proteins.[31] Like the carboxylate-modified polystyrene NPs, the DNA- and siRNA-modified gold NPs are anionic, suggesting the reduced binding in the presence of serum proteins may be due to NP charge rather than composition.

While the conventional view has been that anionic NPs have fewer cellular binding sites and limited uptake compared to cationic NPs,[24] previous reports of the cellular binding of NPs as a function of charge provide a conflicting picture. For example, 18 nm gold NPs modified with a cationic polymer were found to enter adenocarcinoma breast cells (SK-BR-3) to a much greater extent than the same NPs with a negative surface charge.[32] Similar results were obtained for ∼100 nm polymer NPs in adenocarcinoma cervix cells (HeLa).[33] In comparison, the uptake of cerium oxide NPs by adenocarcinoma lung cells (A549) was found to be much greater for anionic NPs.[34] Similarly, a high uptake of anionic 35 nm iron oxide NPs was observed for HeLa cells.[35] While some variation may be due to differences in cell type, similar trends are expected for non-phagocytic cells. On the basis of our results (Figure 2), it is clear that differences in NP binding are highly dependent on the presence or absence of serum proteins: serum proteins enhance the binding of cationic NPs and inhibit the binding of anionic NPs. Interpretation of previous results examining cellular binding and uptake as a function of charge is often difficult because it is not clear from publications if FBS or other serum proteins were present.

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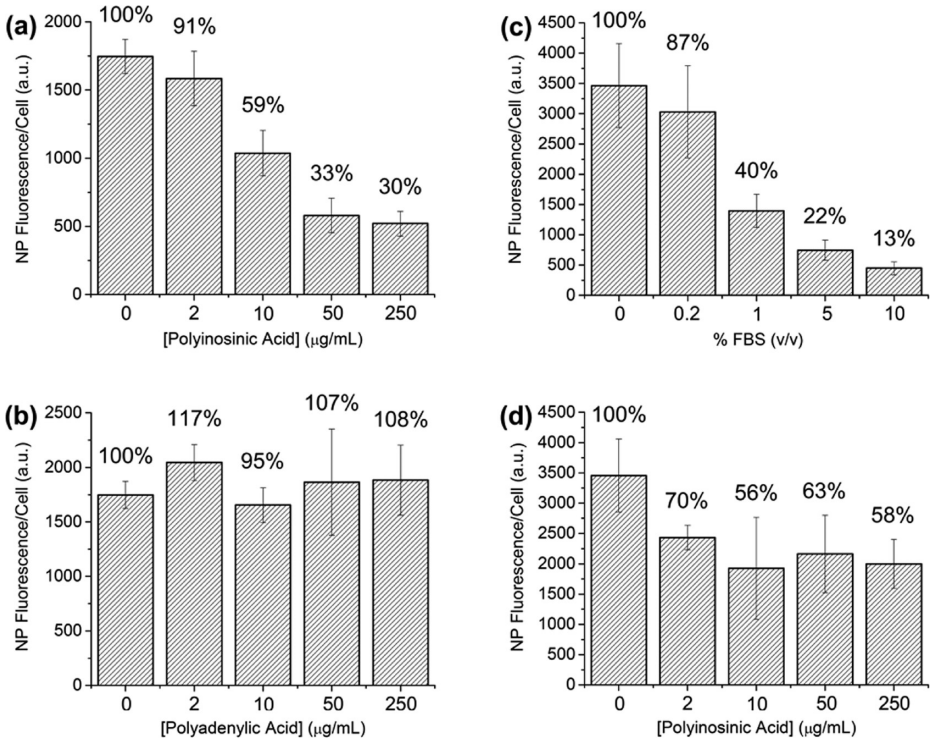

Figure 3. Competition assays showing that protein−NP complexes formed from cationic NPs bind to scavenger receptors, while complexes formed from anionic NPs bind to native protein receptors. (a) Increasing concentrations of polyinosinic acid, a known competitor for scavenger receptors, inhibit the cellular binding of 87 nm amine-modified NPs in MEM supplemented with 10% FBS. The cellular background in the absence of NPs was 11%. Percentages are normalized to NP binding in the absence of polyinosinic acid. (b) Increasing concentrations of polyadenylic acid, which does not bind to scavenger receptors, does not inhibit binding of the 87 nm NPs in MEM supplemented with 10% FBS. (c) The cellular binding of 40 nm carboxylate-modified NPs is inhibited by increasing concentrations of FBS. The cellular background in the absence of NPs was 8%. (d) Increasing concentrations of polyinosinic acid result in minimal inhibition of the cellular binding of 40 nm carboxylate-modified NPs in MEM. Representative flow cytometry data used to generate the bar graphs is included in the Supporting Information (Figure S4).

In addition to the presence of serum proteins, the concentration of NPs is an important factor for cellular binding. Repeating the experiments described above using higher concentrations of NPs, we find that the observed trends no longer hold (Figure S3, Supporting Information). At higher concentrations, cationic NPs can bind to cells in the absence of FBS and anionic NP can bind in the presence of FBS. It is important to note that NP concentration may be one factor responsible for apparently conflicting results in the literature.

Protein−NP Complexes Formed from Cationic NPs Bind to Scavenger Receptors. Although both cationic and anionic NPs form protein−NP complexes, these protein−NP complexes have very different behavior in terms of cellular binding. In the case of the cationic NPs, the formation of the protein−NP complex enhances binding. In comparison, the cellular binding of the protein−NP complex formed with the anionic NPs is inhibited by the presence of serum proteins in solution. This suggests that the protein−NP complex formed from anionic NPs competes with a protein present in FBS for the same binding site, while the protein−NP complex formed from cationic NPs is able to access a different set of cellular receptors.

Blood plasma is a complex mixture of hundreds of distinct proteins, the most abundant (55%) of which is albumin.[8−10] Lower abundance proteins likely exist but are less easily detected. The composition of serum proteins is similar, with the removal of clotting factors. In the case of FBS, the relevant albumin is bovine serum albumin (BSA). Albumin has well-characterized cellular receptors responsible for binding and internalization.[20,21] However, it has been shown that chemical modifications to albumin, including adsorption on the surface of 5 nm gold NPs, alters the binding site of albumin.[36−38] Instead of the cell surface receptors used by albumin, modified albumin binds to gp30 and gp18, which act as scavenger receptors.

To determine if scavenger receptors are responsible for the serum protein-dependent binding of the cationic NPs, we carried out a series of competition assays. Confluent monolayers of BS-C-1 cells were incubated with polyinosinic acid in MEM supplemented with 10% FBS for 20 min. This polyanionic molecule is a known competitor for scavenger receptors.[23,31,36] If the NPs, complexed with serum proteins, bind to scavenger receptors, we expect the addition of polyinosinic acid to compete with the protein−NP complex for binding sites on the cell surface, thereby inhibiting the cellular binding of the NPs.

Flow cytometry was used to measure the NP fluorescence from individual cells (Figure S4, Supporting Information). We found that polyinosinic acid inhibits the binding of the 87 nm cationic NPs in the presence of FBS (Figure 3a). A control experiment with polyadenylic acid, a similar molecule that does not compete for scavenger receptors,[31] shows no competition with the 87 nm NPs (Figure 3b). Similar results were obtained for the 200 nm cationic NPs (Figure S5a, Supporting Information). These results show that scavenger receptors are necessary for the cellular binding of the protein−NP complex formed from cationic NPs.

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Protein−NP Complexes Formed from Anionic NPs Do Not Bind to Scavenger Receptors. Unlike the protein−NP complexes formed from cationic NPs, the cellular binding of the protein−NP complexes formed from anionic NPs is inhibited by the presence of excess serum proteins (Figure 2c and d). This suggests that these protein−NP complexes compete with proteins present in FBS for cellular binding sites. A competition assay using the 40 nm anionic NPs in MEM with increasing concentrations of FBS results in decreased binding (Figure 3c). At 10% FBS, the amount of FBS used in the cellular imaging experiments, only minimal binding is observed. Similar results were obtained for the 200 nm anionic NPs (Figure S5b, Supporting Information). These results demonstrate that the protein−NP complex formed from the 40 nm anionic NPs does not bind to scavenger receptors but instead binds to a native protein receptor.

To determine if the scavenger receptors play any role in the binding of the bare anionic NPs, we carried out a competition assay in MEM, in the absence of FBS, using polyinosinic acid as the competitor (Figure 3d). Compared to the 87 nm protein−NP complex (Figure 3a), cellular binding of the bare 40 nm anionic NPs was minimally inhibited by the presence of polyinosinic acid and lacked any concentration dependence. These results demonstrate that the bare 40 nm anionic NPs do not require binding to scavenger receptors. The specific cellular receptor for the bare 40 nm carboxylate-modified NPs remains unidentified.

CONCLUSIONS

We have found that the binding of NPs to the cell surface in the presence of extracellular serum proteins is highly dependent on the charge of the NP. Although serum proteins adsorb onto the surface of both cationic and anionic NPs in solution, forming a protein−NP complex (Figure 1), these protein−NP complexes display opposite trends in cellular binding. The cellular binding of cationic NPs is enhanced by the presence of serum proteins, while the binding of anionic NPs is inhibited (Figure 2). We have determined that these differences in cellular binding are due to the cellular receptors used by the protein−NP complexes. The protein−NP complex formed from cationic NPs binds to scavenger receptors on the cell surface (Figure 3a). In comparison, the protein−NP complex formed from anionic NPs competes with proteins present in FBS for cellular binding sites (Figure 3c). It is possible that the use of different cellular receptors is due to adsorption of a low abundance protein not detected with gel electrophoresis or differences in the conformation of the adsorbed protein on the NP surface. Differences in protein conformation as a function of charge have been observed previously for gold surfaces.[39]

Our studies focused on NPs of the same composition, polystyrene, modified with chemical groups to control the effective surface charge. While these NPs provide a good model system, it is also worth considering how these results may relate to NPs of other compositions. Binding of serum proteins to NPs appears to be quite general and has been carefully characterized for aluminum oxide NPs,[40] gold NPs and nanorods,[27,28] poly(amidoamine) dendrimers,[41] and quantum dots.[42] The addition of a neutral polymer such as PEG can reduce this nonspecific binding, but the complete inhibition of nonspecific binding remains a challenge.[43−45] This suggests that a wide variety of NPs will be affected by the nonspecific adsorption of serum proteins and that the interaction of these protein−NP complexes will be highly dependent on the initial charge of the NP. The dependence on scavenger receptors for cellular binding of the protein−NP complexes formed from the cationic polystyrene NPs is similar to that observed for 13 nm gold NPs functionalized with DNA or siRNA as well as 5 nm gold NPs complexed with albumin, demonstrating that scavenger receptors are binding sites for NPs of diverse compositions.[23,31,36−38]

NP−cell interactions are governed by a number of factors including NP size, composition, and charge. These interactions are complicated by the presence of extracellular serum proteins that adsorb on the surface of the NP. We anticipate that a direct comparison of the cellular binding of cationic and anionic NPs in the presence of serum proteins will help to reconcile previous results and inform the design of NPs for *in vivo* applications.

ASSOCIATED CONTENT

Supporting Information

Supplemental figures including characterization of the protein−NP complexes, concentration dependent cellular binding of NPs, and raw flow cytometry data are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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