Published in final edited form as:

Biomater Sci. 2013 September 1; 1(9): 975–982. doi:10.1039/C3BM60121H.

Cellular Binding of Anionic Nanoparticles is Inhibited by Serum Proteins Independent of Nanoparticle Composition

Candace C. Fleischer, Umesh Kumar, and Christine K. Payne*

School of Chemistry and Biochemistry and Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, 901 Atlantic Drive, Atlanta, GA, 30332, USA

Abstract

Nanoparticles used in biological applications encounter a complex mixture of extracellular proteins. Adsorption of these proteins on the nanoparticle surface results in the formation of a “protein corona,” which can dominate the interaction of the nanoparticle with the cellular environment. The goal of this research was to determine how nanoparticle composition and surface modification affect the cellular binding of protein-nanoparticle complexes. We examined the cellular binding of a collection of commonly used anionic nanoparticles: quantum dots, colloidal gold nanoparticles, and low-density lipoprotein particles, in the presence and absence of extracellular proteins. These experiments have the advantage of comparing different nanoparticles under identical conditions. Using a combination of fluorescence and dark field microscopy, flow cytometry, and spectroscopy, we find that cellular binding of these anionic nanoparticles is inhibited by serum proteins independent of nanoparticle composition or surface modification. We expect these results will aid in the design of nanoparticles for *in vivo* applications.

Keywords

nanoparticles; serum proteins; quantum dots; colloidal gold; protein corona; opsonization

INTRODUCTION

Nanoparticles (NPs) are increasingly utilized for biological and medical applications.^{1–8} In the course of these applications, NPs will encounter a complex mixture of extracellular proteins. For example, NPs injected into the blood stream are exposed to red and white blood cells, clotting factors, and soluble proteins.⁹ The protein component that remains after blood cells and clotting factors are removed is referred to as serum, which contains hundreds of distinct proteins.^{10,11} Serum proteins readily adsorb onto the surface of NPs, forming a protein corona.^{12,13} This protein corona has been observed on the surface of a wide range of NPs including polymeric NPs,^{14–17} silver nanoclusters,¹⁸ carbon NPs,¹⁹ iron oxide NPs,²⁰ Au nanorods,²¹ and Au NPs.²² Functionalizing the NP surface with polyethylene glycol (PEG) can reduce, but not completely eliminate, this opsonization process.^{18,23–25} The formation of a protein corona on NP surfaces has significant biological implications. Previous reports have determined that adsorbed serum proteins remain bound to the NP during cellular binding and internalization, thereby influencing the cellular receptor and internalization pathway used by the NP.^{15,17,24,26–28} While some of these protein-NP complexes utilize the receptor of the adsorbed protein, others bind to scavenger receptors that are normally used as receptors for denatured or disrupted proteins.^{14,29,30} Advancing

*To whom correspondence should be addressed. christine.payne@chemistry.gatech.edu; Tel: 404-385-3125; Fax: 404-385-6057.

the use of NPs for biological and medical applications requires a better fundamental understanding of how these protein-NP complexes interact with cells.

Given that proteins will adsorb onto the surface of NPs,^{14–22} our goal is to determine the underlying rules that dictate the interaction of the protein-NP complex with the cell. We are especially interested in how adsorbed serum proteins affect the cellular binding of NPs, the first step in the interaction of NPs with cells. We have examined the cellular binding of a collection of anionic NPs with different surface modifications and compositions to determine how a diverse range of NPs interact with cells in the presence of serum proteins. Specifically, we focus on three types of anionic NPs: quantum dots (QDs), colloidal Au NPs, and a biological NP, low-density lipoprotein (LDL). We have previously determined that the cellular binding of anionic carboxylate-modified polystyrene NPs is inhibited by the presence of serum proteins.¹⁴ The free serum proteins in solution compete with the serum protein-polystyrene NP complex for receptors on the cell surface. Our goal is to determine if the trends we observed for polystyrene NPs are also observed for other NPs, especially NPs for biomedical applications.^{4–8}

We first measured the cellular binding of carboxylate-modified CdSe/ZnS QDs using fluorescence microscopy and flow cytometry. QDs are used as cellular sensors and probes due to their bright photoluminescence and size-tunable emission properties.^{6–8} We then repeated these experiments using citrate-modified Au NPs as an example of NPs with a different surface modification and composition. Au NPs have biomedical applications in drug delivery, cellular and tissue imaging, and cancer therapy.^{4,5} Cellular binding was measured with dark field microscopy and UV-Vis spectroscopy. As a point of comparison for synthetic QDs and Au NPs, we also tested the cellular binding of LDL with fluorescence microscopy and flow cytometry. LDL is a <100 nm biological NP that serves as a lipid transporter in the blood stream.³¹ It is composed of cholesteryl ester, cholesterol, phospholipids, and a single molecule of apolipoprotein B-100.³² The use of LDL allows us to determine if NP surface charge plays a similar role for biological NPs. Cationic NPs lacking PEG- or surfactant-modified surfaces are less common for cellular applications and were not probed in these experiments.

We find that binding of anionic carboxylate-modified semiconductor QDs, citrate-modified colloidal Au NPs, and LDL to the cell surface is inhibited by the presence of serum proteins. These results are identical to the trend observed for anionic carboxylate-modified polystyrene NPs.¹⁴ As these NPs vary in surface modification and composition, our results suggest that the initial charge of the NP may be the determining factor in whether NPs will bind to cells in a physiological environment in which serum proteins are present. Considering that cellular binding is the first step in the interaction of NPs with cells, we anticipate that these results will be useful for designing effective NPs for *in vivo* applications.

EXPERIMENTAL METHODS

Nanoparticles (NPs)

Carboxylate-modified CdSe/ZnS quantum dots (QDs, Invitrogen, Q21341MP, emission maximum: 525 nm) were used after sonication for 10 minutes. Citrate-modified Au NPs (Sigma-Aldrich, 753629) were concentrated via centrifugation (10,000g, 10 minutes, 4 °C) and sonicated for 20 minutes prior to cellular binding experiments. Low-density lipoprotein (LDL, Biomedical Technologies, BT-903, 5 mg/mL stock solution) was fluorescently labeled with 1,1'-dioctadecyl-3,3',3'-tetramethylindodicarbocyanine perchlorate (DiD, Invitrogen, D-307) as previously reported,³³ then filtered with a 0.22 µm syringe filter (Fisher Scientific, 09-720-3) immediately prior to use.

Dynamic Light Scattering and Zeta Potential Measurements

A Malvern Zetasizer (Malvern Instruments, Nano-ZS) was used to determine the hydrodynamic diameter (D_h) and zeta potential (ZP) of the NPs. Triplicate measurements were acquired from solutions of NPs at the following concentrations: QDs (80 nM), Au NPs (0.75 pM), and LDL (1 μ g/mL). With the exception of LDL, measurements were made in water. LDL was analyzed in cell culture medium. A reliable D_h value could not be obtained for the QDs due to high absorption of the laser light. Based on analysis of the raw data and literature reports, the D_h of the QDs is approximately 10 nm.³⁴

Cell Culture

African green monkey kidney cells (BS-C-1) were purchased from ATCC. Cells were cultured in a 95% humidity, 5% carbon dioxide atmosphere at 37 °C in minimum essential medium (MEM, Invitrogen, 61100061) with 10% v/v fetal bovine serum (FBS, Invitrogen, 10437028). Cells were passaged every 2–4 days. For fluorescence microscopy and flow cytometry, cells were grown in 35 mm glass-bottom cell culture dishes (MatTek). Cells used for Au NP experiments were seeded in 12-well plates 24 hours prior to cellular binding studies. For dark field imaging, cells were grown on circular glass cover slips (Fisher Scientific, 12-545-100) in 12-well plates.

Fluorescence Microscopy

NPs were incubated with cells at 4 °C in MEM, MEM supplemented with 10% FBS, and MEM supplemented with 10 mg/mL bovine serum albumin (BSA, Fisher, BP1600). This concentration of BSA is equivalent to the total protein concentration in FBS. FBS concentration was calculated from the UV-Vis absorbance value at 276 nm, using an extinction coefficient of 43,824 M⁻¹ cm⁻¹. After incubation with NPs, cells were washed twice with phosphate buffered saline with calcium and magnesium (PBS, Invitrogen, 14040182) to remove unbound NPs and imaged in PBS. Nuclei were stained with 27 μ M 4', 6-diamidino-2-phenylindole dilactate (DAPI, Invitrogen, D3571) in MEM supplemented with 10% FBS at 37 °C for 30 minutes. An inverted epifluorescence microscope (Olympus, IX71) with a 1.20 N.A. 60x water immersion objective (Olympus) and an EMCCD camera (Andor, DU-897) was used to image the fluorescent NPs bound to cells. The following band pass filters were used for imaging: nuclear staining with DAPI (excitation: 387/11; emission: 447/60), QDs (excitation: 480/40; emission: 536/40), and LDL-DiD (excitation: 620/60; emission: 692/40).

Dark Field Microscopy

Cellular binding of Au NPs used the same protocol as fluorescent NPs. After rinsing with PBS, cells were fixed with 4% v/v H₂CO (Thermo Scientific, 28908, 16% v/v stock solution) in PBS for 30 minutes. Images were acquired from cells sealed between the circular cover slip and a second clean cover slip. An inverted microscope (Olympus, IX70) with a 1.35 N.A. 100x oil immersion iris objective (Olympus, UPlanApo) fitted with a dark field condenser (Olympus, U-DCW) was used for dark field imaging.³⁵ A Nikon digital camera (D200) attached to the front port of the microscope was used to image scattered light.

Gel Electrophoresis

QDs (0.8 μ M) were incubated with water, MEM, or MEM supplemented with 10% FBS for 10 minutes before loading onto a 1% w/v agarose gel. Glycerol was added to the QD solutions to aid in well loading. QDs were separated on the gel in Tris-Borate-EDTA buffer (45 mM tris(hydroxymethyl)aminomethane, 45 mM boric acid, and 1 mM

ethylenediaminetetraacetic acid, pH 8.3) for 1 hour and 30 minutes at 90 V with constant voltage. The gel was imaged on a GE Healthcare Typhoon Trio scanner.

Au NPs (1 nM) were incubated in MEM supplemented with 10% FBS for 30 minutes, followed by 5 wash steps via centrifugation (10,000g, 10 minutes, 4 °C), resuspending in H₂O after each step. After the final wash, the Au NP pellet was resuspended in a 6% w/v sodium dodecyl sulfate (SDS) buffer (New England Biolabs, B7703S) to remove the protein corona from the surface of the NPs. Supernatants collected after each wash step were diluted with Laemmli buffer (Boston Bioproducts, BP-110R) and boiled for 5 minutes. The supernatant solutions, Au NP pellets in SDS and water, and FBS alone were loaded onto a polyacrylamide gel with a 5–225 kDa molecular weight marker (Lonza, 50547). The first supernatant was diluted to 1% v/v, and the second supernatant to 10% v/v due to the high protein concentration. A 12% mini-PROTEAN gel (Bio-Rad, 456–8044) run at 130 V and 40 mA was used to separate the proteins. After separation, proteins were stained for 1 hour with Simply Blue Safe Stain (Invitrogen, LC6060), destained overnight, and imaged with a Li-Cor Odyssey imaging system.

Cellular Binding Competition Assays

Cellular binding competition studies of QDs and LDL with 10% FBS and 10 mg/mL BSA were completed using flow cytometry as described previously.¹⁴ Cellular binding competition studies of Au NPs used the same conditions as for dark field microscopy, except that cells were grown in 12-well plates rather than on glass cover slips. To prevent Au NP aggregation, 0.17 nM Au NPs were incubated with 0.5% v/v FBS for 20 minutes to form protein-Au NP complexes before adding to MEM. Competitors, including 10% FBS and 10 mg/mL BSA, were incubated with cells at 4 °C in clear medium for 10 minutes prior to addition of protein-Au NP complexes and were present during NP incubation. After incubation, the supernatant solution above the cell monolayer was transferred to a 96-well plate and absorbance spectra were recorded between 300 and 700 nm with a SpectraMax plate reader (Molecular Devices). The maximum absorbance at 520 nm due to the Au NP plasmon resonance absorption was baseline corrected and subtracted from the absorbance value of Au NPs in the absence of cells. The difference in absorbance is attributed to the Au NPs that remain bound to the cell surface.

Data Analysis

All experiments were performed in triplicate. Mean and standard deviation values are reported for dynamic light scattering, absorbance spectroscopy, and flow cytometry measurements. Significance and p-values were determined using a two-tailed, two-sample independent t-test. All images were analyzed with ImageJ software (<http://rsb.info.nih.gov/ij/>). Comparative images were acquired with the same exposure time and gain. Image brightness and contrast were set to equal values for images for comparison.

RESULTS AND DISCUSSION

The zeta potential (ZP), hydrodynamic diameter (D_h), and biomedical applications of the NPs used in cellular binding experiments are presented in Table 1. To determine the effect of serum proteins on cellular binding, NPs were incubated with cells in minimum essential medium (MEM) without protein and MEM supplemented with 10% v/v fetal bovine serum (FBS). MEM supplemented with 10% FBS is commonly used as a cell culture medium.³⁶ The total protein concentration present in 10% FBS is approximately 10 mg/mL based on the UV-Vis absorbance spectrum. In comparison, physiological serum protein levels are approximately 70 mg/mL.^{10,11,37} The mixture of proteins present in FBS represents the complex biological environment that NPs used for biomedical applications will encounter.

As FBS is a mixture of many different proteins, experiments were repeated using just bovine serum albumin (BSA, 10 mg/mL) the main protein in the FBS mixture.^{10,11,34} This concentration of BSA is equivalent to the total protein concentration present in the 10% FBS experiments. For all NP binding experiments, cells were kept at 4 °C to block cellular internalization and only allow NP binding to the cell surface.^{38–43}

Cellular Binding of Carboxylate-Modified QDs is Inhibited by Serum Proteins

We first confirmed that a protein corona formed on the surface of the anionic QDs, resulting in a protein-QD complex. Previous fluorescence correlation spectroscopy experiments have shown that human serum albumin, the main protein present in human serum,^{10,11,34} adsorbs onto the surface of carboxylate-modified QDs.⁴⁴ We wanted to repeat experiments under our laboratory conditions. QDs (0.8 μM) were incubated in a solution of MEM supplemented with 10% FBS, similar to conditions used in cell culture experiments, for 10 minutes at room temperature. Protein-QD complexes were run on a 1% w/v agarose gel. As a control, QDs incubated in both water and MEM in the absence of protein were run on the same gel. The differing mobilities of QDs in the presence and absence of FBS confirms that QDs exposed to serum proteins form a protein-QD complex (Figure 1). In the absence of protein, QDs show high mobility on the gel (QD + H₂O and QD + MEM). In comparison, the addition of FBS significantly reduces the mobility of the QDs (QD + FBS). The formation of a protein corona on anionic NPs has also been observed for polystyrene NPs,^{14,45} Au nanorods,²¹ and Au NPs.⁴⁶ Most likely, positive residues of the serum proteins interact with the anionic NP surface.²²

To study the effect of serum proteins on the cellular binding of QDs, carboxylate-modified QDs (8 nM) were incubated with BS-C-1 monkey kidney epithelial cells in MEM, MEM supplemented with 10% FBS, and MEM supplemented with 10 mg/mL BSA for 10 minutes. The cells were rinsed twice with PBS prior to imaging to remove QDs that did not bind to the cell surface. In the absence of serum proteins, anionic QDs readily bind to BS-C-1 cells (Figure 2A). In comparison, cellular binding of anionic QDs is blocked by the addition of FBS (Figure 2B). As with FBS, the presence of BSA blocked the cellular binding of QDs (Figure 2C), suggesting that the protein-QD complex competes with free BSA in solution for cellular receptors. Flow cytometry, which provides a high-throughput measurement of cellular fluorescence, was used to quantify the cellular binding of QDs in the presence of serum proteins (Figure 3). Flow cytometry measurements shows that the addition of 10% FBS or 10 mg/mL BSA, protein concentrations used in cell culture, completely inhibits the cellular binding of QDs. The baseline autofluorescence of cells in the absence of QDs is shown for comparison. The quantitative measurements obtained with flow cytometry (Figure 3) are in good agreement with the fluorescence microscopy images (Figure 2).

These results for carboxylate-modified QDs follow the same trend observed for anionic carboxylate-modified polystyrene NPs with hydrodynamic diameters of 60 nm and 236 nm.¹⁴ This suggests that the inhibition of cellular binding of anionic NPs in the presence of serum proteins is independent of NP composition or diameter. In addition, previous work with carbon NPs¹⁹ and anionic silver nanoclusters¹⁸ has shown decreased cellular uptake of NPs in the presence of serum proteins. This may indicate a broader trend, although it is important to note that our experiments measure cellular binding rather than uptake.

Cellular Binding of Citrate-Modified Au NPs is Inhibited by Serum Proteins

As with the QDs, we first confirmed that a protein corona forms on the surface of the citrate-modified Au NPs. Previous research has shown that a protein corona forms on the surface of citrate-modified Au NPs after incubation with a mixture of plasma proteins,⁴⁷ as well as with BSA alone.^{22,48} The formation of a protein corona on the anionic citrate-modified Au

NPs was confirmed by incubating Au NPs (1 nM) with MEM supplemented with 10% FBS for 30 minutes at 4 °C. The NPs were then washed with repeated centrifugation and resuspension in H₂O. The supernatant collected after each wash step was run on a polyacrylamide gel. The final NP pellet was resuspended in a solution containing 6% SDS, a detergent that will remove adsorbed proteins from the NP surface. The first two wash steps result in a high concentration of protein in the supernatant (S1, diluted to 1% v/v, and S2, diluted to 10% v/v, Figure 4). After 4 washes, no protein is detected in the supernatant. The addition of SDS to the Au NP pellet solubilizes any proteins adsorbed on the NP surface resulting in a band at ~66 kDa (NP + SDS). As a control, the addition of water, rather than SDS, does not result in a protein band (NP + H₂O). Based on the molecular weight, it is likely that BSA (66 kDa), the major component of serum (~55%),^{10,11,34} is the main protein adsorbed on the Au NP. These results are in agreement with previous reports that BSA adsorbs onto the surface of citrate-modified Au NPs^{22,46} and borohydride-modified Au NPs.⁴⁹

To image cellular binding, Au NPs (0.34 nM) were incubated with BS-C-1 cells grown on glass cover slips for 30 minutes at 4 °C in MEM or MEM supplemented with 10% FBS. Cells were washed twice with PBS prior to dark field imaging. Cells were fixed to cover slips with 4% formaldehyde and sandwiched between a second glass cover slip for imaging. Using dark field microscopy, we observed that Au NPs bind to cells in the absence of serum proteins and that binding is inhibited in the presence of serum proteins (Figure 5). The decreased binding of citrate-modified Au NPs in the presence of serum proteins is consistent with what we have observed for carboxylate-modified QDs (Figure 2) and carboxylate-modified polystyrene NPs.¹⁴ Control images in the absence of NPs show the scatter from cells in MEM and MEM with 10% FBS (Figure S1).

However, substantial Au NP aggregation is observed in medium without serum, consistent with previous studies.⁴⁶ This allows for the possibility that aggregated Au NPs are able to access a different cellular receptor than individual Au NPs. To ensure that aggregation was not responsible for the difference in cellular binding, we compared the cellular binding of protein-Au NP complexes in the presence and absence of excess FBS. If cellular binding of protein-Au NPs is inhibited by excess FBS, it suggests that the protein-Au NP complexes compete for the same cellular receptors as the free serum proteins in solution.

To measure the cellular binding of non-aggregated protein-Au NP complexes, we used UV-Vis spectroscopy. Absorption spectra of Au NP solutions were acquired before and after incubation with cells. Absorbance at 520 nm, corresponding to the Au NP plasmon resonance, was used as a measure of Au NP concentration. The difference in absorbance before and after incubation with cells was used as a relative measure of Au NPs bound to the cell surface. Representative UV-Vis spectra of Au NPs after incubation with MEM or MEM supplemented with 10% FBS are presented in Figure 6A. Protein-Au NP complexes were formed by incubating Au NPs (0.17 nM) in 0.5% v/v FBS for 20 minutes. The protein-Au NP complexes were then incubated with cells for 30 minutes. The relative differences in absorbance after incubation in MEM, MEM supplemented with 10% FBS (FBS) or MEM supplemented with 10 mg/mL BSA (BSA) were normalized against Au NPs incubated with MEM (Figure 6B). These measurements show that 10% FBS inhibits the cellular binding of Au NPs, similar to the aggregated Au NPs (Figure 5), carboxylate-modified QDs (Figure 2), and carboxylate-modified polystyrene NPs,¹⁴ although the inhibition is less extensive. BSA alone also inhibits the cellular binding of the Au NPs; there is no statistically significant difference between the Au NP binding measured for FBS and BSA. Dark field imaging of single Au NPs in the presence of serum proteins was inconclusive due the decreased scatter of the individual Au NPs (Figure S2).

Cellular Binding of Low-Density Lipoprotein is Inhibited by Serum Proteins

Low-density lipoprotein (LDL) was used as a representative anionic biological NP. Unlike QDs and Au NPs, LDL has a dedicated cellular receptor.⁹ The cellular binding of LDL was observed by fluorescently labeling LDL with DiD, a red lipophilic dye. DiD-labeled LDL (100 µg/mL) was then incubated with BS-C-1 cells at 4 °C for 20 minutes in MEM and MEM supplemented with 10% FBS. In the absence of serum proteins, significant binding of LDL to cells was observed (Figure 7A). In the presence of serum proteins, cellular binding of anionic LDL decreases (Figure 7B). These results demonstrate that the cellular binding of anionic LDL follows the same trends as carboxylate-modified QDs (Figure 2), citrate-modified Au NPs (Figure 6), and carboxylate-modified polystyrene NPs.¹⁴ BSA alone does not inhibit the cellular binding of LDL (Figure 7C). This suggests that lower abundance proteins in FBS, most likely lipoproteins, are responsible for inhibiting LDL binding to the cell surface. These results were quantified with flow cytometry using the same methods as for the carboxylate-modified QDs (Figure 3). The quantitative flow cytometry data is in agreement with the qualitative results from fluorescence microscopy (Figure 7). The presence of 10% FBS (FBS) inhibits the binding of LDL relative to MEM alone (Figure 8, 66% binding), while the addition of 10 mg/mL BSA (BSA) actually increases binding (Figure 8, 171% binding).

CONCLUSIONS

The common theme that emerges from these experiments is that a broad range of anionic NPs can form protein-NP complexes and that the cellular binding of these complexes is inhibited by free serum proteins in solution. The cellular binding of carboxylate-modified QDs, citrate-modified Au NPs, and LDL is inhibited by extracellular serum proteins, similar to previous results for carboxylate-modified polystyrene NPs.¹⁴ While this list of NPs is not comprehensive, it demonstrates that cellular binding trends are consistent for a range of NPs with diverse surface modifications and compositions. In the case of QDs, the competition of QDs with BSA for cellular binding sites suggests that the protein-QD complexes utilize BSA receptors on the cell surface. This may also be true for Au NPs, although the competition is less extensive. For LDL, a low abundance protein within the FBS mixture is likely responsible for the inhibition of cellular binding. NPs used in biological and medical applications will encounter a complex environment of extracellular proteins. Our results show that anionic NPs will likely have inhibited cellular binding, possibly limiting their efficacy. It is expected that these results will inform the design of NPs for use in biological and medical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Prof. Mostafa El-Sayed for use of the dark field microscope and Megan Mackey for her help with dark field imaging. This research was supported by a NIH Director's New Innovator Award (1DP2OD006470) to C. K. P. and a U.S. DoEd Molecular Biophysics and Biotechnology GAANN fellowship (P200A120190) to C. C. F.

References

1. Lavik E, von Recum H. ACS Nano. 2011; 5:3419–3424. [PubMed: 21604811]
2. Wagner V, Dullaart A, Bock AK, Zweck A. Nat Biotechnol. 2006; 24:1211–1217. [PubMed: 17033654]
3. De M, Ghosh PS, Rotello VM. Adv Mater. 2008; 20:4225–4241.

4. Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. *Angew Chem, Int Ed.* 2010; 49:3280–3294.
5. Dreaden EC, Alkilany AM, Huang X, Murphy CJ, El-Sayed MA. *Chem Soc Rev.* 2012; 41:2740–2779. [PubMed: 22109657]
6. Alivisatos AP, Gu W, Larabell C. *Annu Rev Biomed Eng.* 2005; 7:55–76. [PubMed: 16004566]
7. Mattoussi H, Palui G, Na HB. *Adv Drug Delivery Rev.* 2012; 64:138–166.
8. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, Weiss S. *Science.* 2005; 307:538–544. [PubMed: 15681376]
9. Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, JD. *Molecular Biology of the Cell.* Robertson, M.; Adams, R., editors. Garland Publishing Inc; New York: 1994. p. 1164
10. Pieper R, Gatlin CL, Makusky AJ, Russo PS, Schatz CR, Miller SS, Su Q, McGrath AM, Estock MA, Parmar PP, Zhao M, Huang ST, Zhou J, Wang F, Esquer-Blasco R, Anderson NL, Taylor J, Steiner S. *Proteomics.* 2003; 3:1345–1364. [PubMed: 12872236]
11. Anderson NL, Anderson NG. *Mol Cell Proteomics.* 2002; 1:845–867. [PubMed: 12488461]
12. Lynch I, Cedervall T, Lundqvist M, Cabaleiro-Lago C, Linse S, Dawson KA. *Adv Colloid Interface Sci.* 2007; 134–135:167–174.
13. Walczyk D, Bombelli FB, Monopoli MP, Lynch I, Dawson KA. *J Am Chem Soc.* 2010; 132:5761–5768. [PubMed: 20356039]
14. Fleischer CC, Payne CK. *J Phys Chem B.* 2012; 116:8901–8907. [PubMed: 22774860]
15. Doorley GW, Payne CK. *Chem Commun.* 2011; 47:466–468.
16. Cedervall T, Lynch I, Foy M, Berggard T, Donnelly SC, Cagney G, Linse S, Dawson KA. *Angew Chem, Int Ed.* 2007; 46:5754–5756.
17. Ehrenberg MS, Friedman AE, Finkelstein JN, Oberdörster G, McGrath JL. *Biomaterials.* 2009; 30:603–610. [PubMed: 19012960]
18. Shang L, Dörlich RM, Trouillet V, Bruns M, Nienhaus GU. *Nano Res.* 2012; 5:531–542.
19. Zhu Y, Li W, Li Q, Li Y, Li Y, Zhang X, Huang Q. *Carbon.* 2009; 47:1351–1358.
20. Jedlovsky-Hajdú A, Bombelli FB, Monopoli MP, Tombácz E, Dawson KA. *Langmuir.* 2012; 28:14983–14991. [PubMed: 23002920]
21. Alkilany AM, Nalaria PK, Hexel CR, Shaw TJ, Murphy CJ, Wyatt MD. *Small.* 2009; 5:701–708. [PubMed: 19226599]
22. Brewer SH, Glomm WR, Johnson MC, Knag MK, Franzen S. *Langmuir.* 2005; 21:9303–9307. [PubMed: 16171365]
23. Gref R, Lück M, Quellec P, Marchand M, Dellacherie E, Harnisch S, Blunk T, Müller RH. *Colloids Surf, B.* 2000; 18:301–313.
24. Walkey CD, Olsen JB, Guo H, Emili A, Chan WCW. *J Am Chem Soc.* 2011; 134:2139–2147. [PubMed: 22191645]
25. Kim HR, Andrieux K, Delomenie C, Chacun H, Appel M, Desmaële D, Taran F, Georgin D, Couvreur P, Taverna M. *Electrophoresis.* 2007; 28:2252–2261. [PubMed: 17557357]
26. Doorley GW, Payne CK. *Chem Commun.* 2012; 48:2961–2963.
27. Monopoli MP, Aberg C, Salvati A, Dawson KA. *Nat Nanotechnol.* 2012; 7:779–786. [PubMed: 23212421]
28. Lesniak A, Fenaroli F, Monopoli MP, Åberg C, Dawson KA, Salvati A. *ACS Nano.* 2012; 6:5845–5857. [PubMed: 22721453]
29. Schnitzer JE, Bravo J. *J Biol Chem.* 1993; 268:7562–7570. [PubMed: 8463286]
30. Patel PC, Giljohann DA, Daniel WL, Zheng D, Prigodich AE, Mirkin CA. *Bioconjugate Chem.* 2010; 21:2250–2256.
31. Brown MS, Goldstein JL. *Science.* 1986; 232:34–47. [PubMed: 3513311]
32. Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, JD. *Molecular Biology of the Cell.* Robertson, M.; Adams, R., editors. Garland Publishing Inc; New York: 1994. p. 621–622.
33. Humphries WH, Fay NC, Payne CK. *Integr Biol.* 2010; 2:536–544.
34. Cady NC, Strickland AD, Batt CA. *Mol Cell Probes.* 2007; 21:116–124. [PubMed: 17084590]

35. Kang B, Mackey MA, El-Sayed MA. J Am Chem Soc. 2010; 132:1517–1519. [PubMed: 20085324]
36. Freshney, RI. Culture of Animal Cells. John Wiley & Sons, Inc; Hoboken: 2005. p. 123-125.
37. Adkins JN, Varnum SM, Auberry KJ, Moore RJ, Angell NH, Smith RD, Springer DL, Pounds JG. Mol Cell Proteomics. 2002; 1:947–955. [PubMed: 12543931]
38. Pastan I, Willingham M. Science. 1981; 214:504–509. [PubMed: 6170111]
39. Szymanski, CJ.; Yi, H.; Liu, JT.; Wright, ER.; Payne, CK. Nanobiotechnology Protocols. Rosenthal, SJ.; Wright, DX., editors. Humana Press; New York: 2012.
40. Callaini G, Dallai R, Riparbelli MG. Exp Cell Res. 1991; 194:316–321. [PubMed: 1709105]
41. Watts RG, Howard TH. Cell Motil Cytoskel. 1992; 21:25–37.
42. Goldenthal KL, Pastan I, Willingham MC. Exp Cell Res. 1984; 152:558–564. [PubMed: 6144562]
43. Harding C, Heuser J, Stahl P. J Cell Biol. 1983; 97:329–339. [PubMed: 6309857]
44. Rocker C, Potzl M, Zhang F, Parak WJ, Nienhaus GU. Nat Nanotechnol. 2009; 4:577–580. [PubMed: 19734930]
45. Gessner A, Lieske A, Paulke BR, Müller RH. J Biomed Mater Res, Part A. 2003; 65A:319–326.
46. Casals E, Pfaller T, Duschl A, Oostingh GJ, Puentes V. ACS Nano. 2010; 4:3623–3632. [PubMed: 20553005]
47. Dobrovolskaia MA, Patri AK, Zheng J, Clogston JD, Ayub N, Aggarwal P, Neun BW, Hall JB, McNeil SE. Nanomed-Nanotechnol. 2009; 5:106–117.
48. Dominguez-Medina S, McDonough S, Swanglap P, Landes CF, Link S. Langmuir. 2012; 28:9131–9139. [PubMed: 22515552]
49. Chakraborty S, Joshi P, Shanker V, Ansari ZA, Singh SP, Chakrabarti P. Langmuir. 2011; 27:7722–7731. [PubMed: 21591651]

Figure 1.

Formation of a protein corona on carboxylate-modified QDs was confirmed using gel electrophoresis (1% w/v agarose). To form the corona, QDs (green) were incubated with MEM supplemented with 10% FBS (QD + FBS). As a control, QDs were incubated in water (QD + H₂O) and MEM (QD + MEM) in the absence of protein.

Figure 2.

Fluorescence microscopy images of carboxylate-modified QDs (green) bound to BS-C-1 cells. Cell nuclei are stained with DAPI (blue). QDs were incubated with cells for 10 minutes at 4 °C in (A) MEM, (B) MEM supplemented with FBS, and (C) MEM supplemented with BSA.

Figure 3.

Flow cytometry was used to measure cellular binding of QDs in MEM, MEM supplemented with FBS (FBS), MEM supplemented with BSA (BSA), and cells in the absence of quantum dots (Cells only). Results were normalized against QD binding in MEM.

Figure 4.

Formation of a protein corona on the surface of citrate-modified Au NPs after incubation in MEM supplemented with 10% FBS. Au NPs were washed via centrifugation five times and supernatants (S) were analyzed with SDS-PAGE. S1 was diluted to 1% and S2 was diluted to 10% v/v due to the high protein concentration. Protein is no longer visible in the supernatant after 4 wash steps. The protein corona is removed from the surface of the NP with SDS (NP + SDS). In the absence of SDS (NP + H₂O), no protein is evident on the gel. FBS is shown for comparison. Molecular weight (MW) marker values are 225, 150, 100, 75, 50, 35, 25, 15, 10, and 5 kDa.

Figure 5. Dark field microscopy images of citrate-modified Au NPs (yellow) bound to BS-C-1 cells after incubation for 30 minutes at 4 °C in (A) MEM and (B) MEM supplemented with FBS. Images of cells without Au NPs are presented in Figure S1, showing scatter due to cells alone.

Figure 6.

Cellular binding of protein-Au NP complexes was measured using the absorption spectra of Au NPs before and after incubation with the cells. (A) Representative difference spectra show the relative cellular binding of Au NPs following incubation with cells in the presence (FBS) or absence (MEM) of serum proteins. The difference spectra of cells in the absence of Au NPs is shown for comparison (Cells only). (B) Binding of protein-Au NP complexes in MEM, MEM supplemented with FBS, and MEM supplemented with BSA. Absorbance of medium incubated with cells, but without NPs, was negligible. Binding was normalized to 100% for Au NPs incubated with cells in MEM (* $p < 0.05$; ** $p < 0.01$; there was no statistically significant difference between FBS and BSA).

Figure 7. Fluorescence microscopy images of LDL fluorescently labeled with DiD (red) bound to BS-C-1 cells. Cell nuclei are stained with DAPI (blue). LDL-DiD was incubated with cells for 20 minutes at 4 °C in (A) MEM, (B) MEM supplemented with FBS, and (C) MEM supplemented with BSA.

Figure 8.

Flow cytometry was used to measure the cellular binding of LDL particles in MEM, MEM supplemented with FBS (FBS), MEM supplemented with BSA (BSA), and cells in the absence of LDL (Cells only). Binding was normalized against LDL binding in MEM (* $p < 0.05$; *** $p < 0.001$).

Table 1

Properties of nanoparticles used in cellular binding experiments

NP	Surface Modification	ZP (mV)	D _h (nm)	Applications
QD	carboxylate	-45.2 ± 1.0	N/A ^a	Cellular imaging and sensing, FRET probes ⁶⁻⁸
Au	citrate	-17.7 ± 2.9	39.3 ± 2.2	Drug delivery, <i>in vivo</i> imaging, photothermal therapy ^{4,5}
LDL ^b	N/A	-15.8 ± 2.1	68.0 ± 20.6	Lipid transport ³¹

^aD_h ~10 nm, see Experimental Methods.

^bMeasurements carried out in Minimum Essential Medium.

ZP, zeta potential; D_h, hydrodynamic diameter; QD, quantum dot; FRET, fluorescence resonance energy transfer; LDL, low-density lipoprotein