Adsorption and Unfolding of a Single Protein Triggers Nanoparticle Aggregation

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Supporting Information

ABSTRACT: The response of living systems to nanoparticles is thought to depend on the protein corona, which forms shortly after exposure to physiological fluids and which is linked to a wide array of pathophysiology. A mechanistic understanding of the dynamic interaction between proteins and nanoparticles and thus the biological fate of nanoparticles and associated proteins is, however, often missing mainly due to the inadequacies in current ensemble experimental approaches. Through the application of a variety of single molecule and single particle spectroscopic techniques in combination with ensemble level characterization tools, we identified different interaction pathways between gold nanorods and bovine serum albumin depending on the protein concentration. Overall, we found that local changes in protein concentration influence everything from cancer cell uptake to nanoparticle stability and even protein secondary structure. We envision that our findings and methods will lead to strategies to control the associated pathophysiology of nanoparticle exposure in vivo.

KEYWORDS: protein corona, nanorods, superlocalization microscopy, correlation spectroscopy, surface plasmon

The interaction of nanoparticles with living organisms has received growing attention in the past decade due to the development of novel therapeutic and diagnostic tools, and the growing concerns regarding the safety of nanomaterials in vivo. It is understood that nanoparticles adsorb proteins in biological fluids forming a “protein corona”, which influences the nanoparticle physicochemical properties and subsequent interactions with the physiological environment. Both the possibility that in vivo administration of therapeutic nanoparticles can disturb the structural integrity of proteins and the current knowledge of the direct link between denatured proteins and severe neurodegenerative diseases suggest that nanotherapeutic platforms potentially pose a health risk. Therefore, understanding the interaction of proteins with nanoparticles on a molecular level is paramount for the efficient and safe application of engineered nanoparticles.

The quantification and identification of the protein corona composition for different types of nanoparticles with varying surface chemistries have been undertaken. Ex situ ensemble techniques such as gel electrophoresis coupled with mass spectrometry have been widely used to characterize the chemical identity and abundance of constituent corona proteins after separation from the nanoparticles. Less disruptive, complementary optical spectroscopy approaches can be used to study the protein−nanoparticle complex in situ and were able to determine the thermodynamics of protein binding as well as to provide insight into the interactions between protein coated nanoparticles and cells. For instance, the micromolar affinity of serum albumin to colloidal nanoparticles resulting in a protein monolayer at physiological concentrations and enhanced colloidal stability in plasma have been revealed by a combination of UV−vis and fluorescence correlation spectroscopic techniques. Payne and co-workers have used super-resolution optical imaging under in vitro conditions, and demonstrated that what cellular receptors actually screen are the adsorbed proteins and not the initially synthesized nanoparticles themselves. Spectroscopic methods are ideally suited to characterize changes in protein structure...
when bound to a nanoparticle surface. The structural integrity of the corona proteins, which domains of the protein bind and which ones potentially change their structure, has been studied by using far- and near-UV circular dichroism (CD), IR, and Raman spectroscopy.

While the protein-nanoparticle community has begun to transition from the mere identification of adsorbed proteins to the understanding of the physiological responses that can arise due to the presence of the protein corona, it is still not fully understood how protein adsorption affinities, relative concentrations, and surface chemistries are ultimately linked to the corona composition and to possible structural changes of the proteins. A mechanistic bridge between these aspects is lacking due to the low spatiotemporal resolution and ex situ requirements of most current techniques. Ensemble analytical techniques that assume quasi-equilibrium conditions with exchange between free and bound proteins have furthermore led to controversial results.

Here, we have used a combined approach of ensemble and single protein/single nanoparticle methods to answer the following questions about the nanoparticle protein corona: Is thermodynamic equilibrium a valid concept for the protein corona as local changes in protein concentrations occur? How does protein adsorption influence protein chemistry? How is nanoparticle stability altered? Finally, how might equilibrium and nonequilibrium protein/nanoparticle interactions influence physiological outcomes? Mechanistic understanding of the physicochemical properties of bovine serum albumin (BSA) adsorbed to cationic-ligand functionalized gold nanorods (AuNRs) is studied here as the experimental system. While an ensemble protein adsorption isotherm implicates the adsorption of several proteins to the nanoparticle surface, single protein/single nanoparticle interactions visualized through superlocalization imaging reveal that in fact only one protein is irreversibly adsorbed at a time. The seemingly conflicting findings are resolved through surface plasmon coupled CD spectroscopy, and demonstrate that the local changes in protein concentration affect the colloidal stability of nanoparticles, protein secondary structure and eventually cellular uptake of nanoparticles.

RESULTS AND DISCUSSION

When nanoparticles are preincubated in BSA at lower than physiological concentrations, properties such as uptake by MCF-7 cancer cells, BSA adsorption, and nanoparticle aggregation are strongly influenced (Figure 1). Cationic mercaptoundecyltrimethylammonium bromide coated AuNRs (MUTAB-AuNRs) incubated in 10% fetal bovine serum (FBS) are uptaken by MCF-7 cancer cells (Figure 1a,b). However, with preincubation of the MUTAB-AuNRs in 1% (w/v) BSA, the uptake is increased 3-fold (Figure 1b). This increase is not due to MUTAB-AuNRs adhered to the outside of the cells as the cells are washed several times with PBS following a previously published protocol. As the most abundant serum protein, BSA is present in higher concentrations in FBS than 1% (w/v). Preincubation of MUTAB-AuNRs with 1% (w/v) BSA, however, seems to influence their interaction with cancer cells and causes increased uptake. It is thus important to...
understand if and how the BSA-based protein corona is protein dependent. **In situ** ensemble, single molecule, and single particle spectroscopies are used to address these issues.

Luminescence correlation spectroscopy provides strong support that the BSA protein corona that forms on MUTAB-AuNRs is concentration dependent (Figure 1c). At high protein concentrations (20 μM), the increase in measured hydrodynamic radius matches the dimensions of native BSA53 and hence implies the formation of a protein monolayer, consistent with earlier reports of BSA monolayer formation on nanoparticles (Table S1).15,54,55 In contrast, at lower BSA concentrations (2 nM), the luminescence correlation decay shifts to longer average decay times, indicating that surprisingly the nanoparticle–protein complex has become even larger than what is observed under the high BSA concentration conditions. Most importantly, the data could not be fit to an equilibrium adsorption model, suggesting nonequilibrium behavior, consistent with nanoparticle aggregation as verified further below.

Further support for the strong deviation between low and high BSA concentrations is found in the UV−vis spectra (Figure 1d). At high BSA concentrations, the extinction spectrum of the MUTAB-AuNRs is slightly red-shifted, due to a change in dielectric environment and consistent with equilibrium formation of a stable BSA monolayer. However, at low BSA concentrations, the decreased intensity and resonance shift of the plasmon resonance indicate that the MUTAB-AuNRs aggregate in the presence of low BSA concentrations.

The combination of our results on cancer cell uptake, protein adsorption, and nanoparticle colloidal stability strongly suggest that local protein concentrations must be considered when assessing corona chemistry and its influence on nanoparticles’ ultimate physiological fate, as suggested previously.11,20 Of particular interest in Figure 1 is the case of low protein concentrations. Low protein-to-nanoparticle ratios can, for example, occur under controlled preincubation conditions after injection into a living organism and upon accumulation in cellular compartments. Thus, we examine more closely the structure of BSA and nanoparticles when they interact at low concentrations and suggest how these processes lead to the ultimate fate of the protein–nanoparticle colloid.

**Figure 2.** Single molecule imaging of BSA interactions with nanomaterials of varying geometries (sphere, wire, rod) and surface chemistries (cationic MUTAB, cationic NH2-PEG-SH, anionic citrate). Images show nanomaterial highlighted with dashed red outline (left) before and (right) after 2 nM Alexa-BSA is introduced. Images are binned, ranging from 10 to 100 frames. These results show that BSA binding occurs independent of the nanoparticle geometry. Furthermore, a different surface chemistry also facilitates binding as long as the surface charge remains positive. No significant adsorption is seen for negative charged citrate capped nanoparticles at this BSA concentration.
and BSA as example nanoparticle supports and serum proteins, respectively.

Single molecule analysis of the fluorescence imaging data reveals that only one BSA protein binds irreversibly to each MUTAB-AuNR (Figure 3). BSA adsorption onto single MUTAB-AuNRs is identified by colocalization of dye fluorescence and intrinsic AuNR luminescence (Figure 3a, Figures S1–S3 and Video S1). Scanning electron microscopy (SEM) image analysis confirms that 99% of the analyzed BSA adsorption events occur at single MUTAB-AuNRs (Figure S4) with little nonspecific BSA interaction with the substrate because of surface passivation (Figure S5).

Although BSA in its native form is smaller than a AuNR, we find no evidence for multiple BSA adsorption by analyzing fluorescence time transients (Figure 3b,c). Protein adsorption is observed as an increase in fluorescence intensity, followed by a stepwise decrease as each of the dye molecules on the protein photobleaches (Figure 3b). Photobleaching step and intensity distribution analyses are commonly used in single molecule experiments to detect single vs oligomer states of proteins, including proteins with multiple dyes. The number of photobleaching steps identified by an established step-finding algorithm is thus a direct measure of the BSA molecules per AuNR (Figure 3b, line). Analysis for 86 AuNRs yields an average of 3.2 ± 1.2 photobleaching steps (Figure 3c), matching the single protein labeling density indicated by the supplier (Molecular Probes, A34785). Further controls involve eliminating the presence of simultaneous photobleaching of multiple dyes (Figure 3d), reversible protein desorption/readsorption (Figure S6), or dye fluorescence quenching close to the AuNRs (Video S2). In particular, Figure 3d confirms that despite the labeling with 3 dyes a BSA molecule does not behave as a multichromophoric system capable of simultaneous off/on blinking of all dyes due to energy transfer.

The adsorption of single BSA proteins to single MUTAB-AuNRs is irreversible on a time scale of 8 h as demonstrated by a constant percentage of AuNR-BSA complexes while continuously rinsing with buffer solution without proteins (Figure 4). It is not clear, however, if this observation of only one protein adsorbing to each immobilized MUTAB-AuNR extends to the solution case. The exposed AuNR surface area is about twice as large in solution, but the protein-to-nanoparticle ratio is only ~26, as ~75 pM nanorods are exposed to 2 nM BSA. It is not trivial to quantify this ratio for MUTAB-AuNRs immobilized on a substrate, but we estimate this number to be orders of magnitude larger given that 2 nM BSA (~10^8 BSA proteins/μL) is flowed over single nanorods spaced out at least
1 μm from each other at a flow rate of 5 μL/min for 15 min. Regardless of the experimental geometry (i.e., free MUTAB-AuNRs in solution vs immobilized on a surface), there is space for many dozens of proteins to bind to each AuNR (width = 20 nm, length = 58 nm; resulting surface area of ∼3000 nm²), assuming a native BSA conformation that can be approximated by an equilateral triangular prism with 2 triangular facets of ∼28 nm² and 3 smaller rectangular sides of ∼12 nm. The only hypothesis that explains how the strong, irreversible adsorption of single (or few) BSA molecules can lead to the results shown in Figure 1 is if BSA is undergoing structural changes upon adsorption.

Using CD spectroscopy and subdiffraction localization microscopy, we find that BSA undergoes an unprecedented large loss in α-helical structure when adsorbed to MUTAB-AuNRs (Figure S). BSA is primarily α-helical in secondary structure. Therefore, increases in the negative peaks at 209 and 222 nm and decreases in the positive peak at 192 nm in the UV CD spectrum indicate a loss of BSA α-helical structure in the presence of MUTAB-AuNRs (Figure 5a). Similar behavior is observed for MUTAB-coated Au nanospheres (Figures S7 and S8), in contrast to citrate-capped Au nanospheres or in the presence of only MUTAB ligand (Figures S9, S10a, S11). From the UV CD signal, we calculate the loss of protein order using a secondary structure analysis to be 20 ± 9%, similar to results obtained on cationic-functionalized silica particles. This analysis, however, provides only a low estimate because of unbound native BSA in solution. After centrifugation and removal of the supernatant protein binding events are imaged. Most of the available nanowire surface area is covered by BSA (Figure 2). Because of this high coverage, the smallest distance between nearest-neighbor proteins is determined by cross-correlating the intensity transients of neighboring pixels (Figures S13 and S14). Such analysis, compared to superlocalization mapping of individual fluorophores, is less affected by possible distortions in the point spread functions of the protein dye labels due to their positions and orientations on a plasmonic substrate. Figure 5c shows the most likely locations of seven individual BSA proteins adsorbed onto a large MUTAB-Au nanowire identified with high confidence. The average distance between neighboring unfolded BSA proteins is larger than 200 nm and explains why only a single BSA molecule can bind to the available surface area of the smaller MUTAB-AuNRs. On the basis of the change in adsorbed protein structure, it is possible to explain the nanoparticle aggregation at low BSA concentrations (Figure 1d).

An unfolded BSA corona on MUTAB-AuNRs causes AuNR aggregation (Figure 6). MUTAB-AuNRs preincubated with BSA form aggregates with immobilized MUTAB-AuNRs that were similarly preincubated with BSA. Figure 6a shows a cartoon of the experiment conditions, in which large aggregates are formed on the substrate as observed by SEM (Figure 6b, insets). Particle size analysis quantifies a large increase in both the number of structures (single AuNRs and aggregates) and distribution of surface areas for the samples with substrate and solution BSA/MUTAB-AuNR in comparison to samples consisting of only one of the components (Figure S15 and Table S2).

Consistent with irreversible BSA binding, we find no evidence for protein aggregates in solution (Figure S11).
Further, anionic citrate-capped Au nanospheres, which adsorb BSA but do not induce unfolding, do not aggregate (Figure S9). Most interestingly, fibrinogen and globulin cause aggregation of MUTAB-AuNRs not only at low protein concentration, but also at physiological conditions, potentially making the behavior of BSA unique among the most abundant serum proteins (Figure S16). We therefore hypothesize that protein unfolding on a highly charged nanoparticle surface is in general a favorable process, but at the high physiological concentrations of BSA, its designed biological function as a solubilizing and transporting agent dominates due to complete monolayer coverage. This aspect will be subject of future work. It should be mentioned that, while this work was under review, a similar counterintuitive relationship between protein concentration and nanoparticle aggregation was published by Cedervall and co-workers.70 Despite using negatively charged polystyrene nanoparticles suspended in cow serum, they found that IgG can induce nanoparticle aggregation at low protein concentrations, while at high concentrations, a protein monolayer prevents the formation of large nanoparticle aggregates.

**CONCLUSIONS**

On the basis of our experimental results obtained from a variety of single molecule/particle and ensemble techniques, we propose a nonequilibrium mechanism in which irreversible protein adsorption occurs at low protein-to-nanoparticle ratios and is followed by BSA unfolding (Figure 6c). In turn, unfolded BSA–BSA interactions drive the nanoparticle aggregation process. Although protein-induced nanoparticle aggregation28,43 has been reported in the literature, we believe we have provided a mechanistic explanation of how this process can happen under specific conditions. Furthermore, we have directly addressed the questions posed above. First, although we often model protein adsorption as a dynamic equilibrium, it is clear that under certain circumstances, the interaction between serum proteins and nanoparticles can undergo strong nonequilibrium processes such as unfolding and aggregation. Our results more broadly imply that modeling of protein adsorption on nanoparticles using commonly employed equilibrium binding constants may describe at best a pseudoequilibrium occurring at large protein to nanoparticle ratios. Next, the extreme changes detected in MUTAB-AuNR size in the presence of low concentrations of BSA are consistent with unfolding of single (or few) proteins on single AuNRs followed by aggregation driven by unfolded BSA–BSA interactions. As for the last and most difficult question, we present strong support of a hypothesis in which the interaction of MUTAB-AuNRs or other therapeutic nanoparticles with even small amounts of plasma proteins, along with their subsequent change in secondary structure, could strongly influence their ultimate fate, be that for good or bad. Much further work is required to test this hypothesis. Regardless, these results show how the protein-to-nanoparticle ratio influences the physical chemistry of the protein corona, and that achieving a single protein level of mechanistic insight will deepen our understanding of the connections between protein corona composition, structure and in vivo physiological pathways.

**MATERIALS AND METHODS**

**Gold Nanoparticles.** Commercially available AuNRs (A12-25-750, Nanopartz, Loveland, CO, USA) suspended in water and coated with cetyltrimethylammonium bromide (CTAB) were functionalized with MUTAB, a cationic ligand that covalently attaches to the gold surface via a sulfur bond. Excess CTAB was removed by centrifugation of the stock solution for 10 min at 7500 rpm. The AuNRs were then resuspended in 1 mg/mL MUTAB in Millipore H2O (>1 M Ω) and placed overnight in a water bath at 35 °C. Excess MUTAB was removed by centrifugation at 7500 rpm for 10 min and replaced with Millipore H2O for storage. We estimate that the final concentration of free MUTAB in solution after this centrifugation step was less than 0.01 mg/mL. The MUTAB-AuNR solutions were positively charged ($\zeta = 35 \pm 5$ mV) at pH 7.2 according to zeta-potential measurements (Malvern Zen 3600). Further analyses of the MUTAB-AuNRs in the buffer conditions of the experiments (20 mM HEPES, 20 mM NaCl in Molecular Biology Grade H2O) by UV/vis spectroscopy (Figures S17 and S18) and by transmission electron microscopy (TEM) (Figure S19) are included in the Supporting Information.

Commercially available citrate-coated gold nanospheres (AuNPs) with a nominal diameter of 50 nm were purchased from BBI solutions (Cardiff, U.K.). Sizing performed by the manufacturer using TEM showed that their actual size is 48 ± 4 nm (Batch # 16659, ~75 pM concentration based on the mass of gold per milliliter used for the
Gold nanowires (Au nanowires, diameter ~70 nm, length 1–10 μm) were synthesized using a modified three-step seeding synthesis originally developed for AuNRs. Seed particles were prepared by a rapid reduction of HAuCl₄ (gold precursor) using NaBH₄ (reducing agent). These particles were then grown in a solution containing HAuCl₄ ascorbic acid, and CTAB. The presence of ascorbic acid reduces the gold salt from its Au(III) to a stable Au(I) state. Further addition of seeds to this solution induces an autocatalytic reaction of Au(I) that enlarges the seed particles. While this method would typically yield nanowires with an aspect ratio smaller than 10, aspect ratios larger than 100 can be achieved when the reaction takes place in an acidic environment (pH ~ 2) and less seeds are added to the growth solution. CTAB on the as-synthesized Au nanowires was replaced with MUTAB. The CTAB-Au nanowires sedimented at the bottom of the vial after 1–2 h at room temperature without mixing or shaking. Carefully, CTAB was removed out of solution without removing the nanowires. An equal volume of MUTAB (1 mg/mL in Millipore H₂O) was added to fill the volume left by removed CTAB. The solution was mixed using a micropipette and placed overnight in a water bath at 35 °C. The MUTAB-Au nanowires were then resuspended in solution by gently shaking the vial, and then left untouched for 1–2 h at room temperature to allow sedimentation. Finally, excess MUTAB was removed out of solution (carefully without removing the nanowires), and an equal volume of Millipore H₂O was added to fill the volume left by excess MUTAB.

**Cell Culture.** BSA (7.5% in DBPS), penicillin–streptomycin (10 000 U–10 mg/mL), HCl (Trace Metals Grade) and HNO₃ (Trace Metals Grade) were purchased from Sigma-Aldrich. MCF-7 cells were acquired from ATCC (HTB-22). EMEM (with EBSS and L-BSA (7.5% in DBPS), penicillin–streptomycin) of concentrations used. Results of correlation spectroscopy experiments before and after BSA binding at different concentrations are shown in Table S1.

**Superlocalization Single Molecule Microscopy.** Samples containing single immobilized MUTAB-AuNRs (Figures 2 and 3) and MUTAB-AuNPs (Figure 2) were prepared by dropcasting ~40 μL of ~12.5 pM of nanoparticle solutions (1:6 dilution of the concentration used for luminescence correlation spectroscopy experiments) on microscope coverslips passivated with unlabeled BSA (see caption of Figure S5 for details on the passivation procedure). The droplet was then dried for 10 min at room temperature, and the coverslips were rinsed with 1 mL of Millipore H₂O and gently dried with nitrogen gas. A home-built total internal reflection wide field microscope with 637 nm excitation (Coherent OBIS-FP 637 LX) as described elsewhere was used with a custom flow chamber (1 mm height, elliptical opening of 12 × 5 mm; 43018C, Grace BioLabs) placed over the sample containing immobilized MUTAB-nanoparticles. A solution of 2 nM Alexa647-labeled BSA (A34785, Molecular Probes, 3 dyes/protein) was flowed over the sample at 5 μL/min using a Genie Plus flow system (Kent Scientific). This flow rate does not impart strong forces on protein-binding site interactions and results in effectively negligible net directed diffusion due to flow. The sample was allowed to equilibrate for 15 min with protein flow before measuring, ensuring that an excess of proteins is exposed to each nanoparticle (2 nM BSA/L × 5 μL/min × 15 min × Nₕ = 9 × 10¹⁰ BSA molecules compared to ~10 particles/5 μm² in Figure S3 × 60 μm² area of sample = 12 × 10¹⁰ particles/sample). Data was collected at an incident excitation intensity of 5 mW/cm², an integration time of 100 ms, frame rate of 7.5 Hz, and electron multiplying gain of 300. Analysis was possible at the superlocalization level, as the fluorescent BSA can be selectively observed only when adsorbed to the interface, and was unobservable by motion blur when freely diffusing (D ~ 60 μm²/s). Increases in signal-to-noise ratio of each frame, identification of adsorbed BSA, and superlocalization analysis of the location by radial symmetry fitting was performed using MATLAB 2011b as described previously. Samples containing single immobilized MUTAB-Au nanowires (Figures 2, 5c, and S14) were prepared using the same drying procedure, but because the

**Luminescence Correlation Spectroscopy.** Luminescence of MUTAB-AuNRs was recorded with a home-built confocal microscope described elsewhere. The instrument is based on an inverted epifluorescence microscope (Observer.D1, Zeiss) equipped with a 638 nm diode laser (CUBE, Coherent). The laser light was collimated and a n 50 μm diameter pinhole (Thorlabs) placed at the focal plane of the microscope. The luminescence signal was detected by a single element diode detector (PDM 50ct, Micro-Photon-Devices) and processed by a home-built photon counting module (LabView). All measurements were recorded at a controlled laboratory temperature of 20 ± 1 °C, and using an excitation power of 1.5 × 10⁶ mW/cm² at the sample. This excitation power ensured minimal heating and negligible autofluorescence background of unlabeled BSA (Figure S20). For protein concentration dependent measurements as summarized in the table, the excitation power ensured minimal heating and negligible autofluorescence background of unlabeled BSA (Figure S20).

**Table S1.** ~75 pM MUTAB-AuNR solutions with varying concentrations of BSA (Catalog #A7906, Sigma-Aldrich; BSA concentrations, 0.1, 0.5, 1, 1.5, 2, 3, 4, 5, 10 nM; BSA/AuNR ratios, 1.33, 6.66, 13.33, 20, 26.66, 40, 53.33, 66.66, 133.33) were prepared by mixing equal volumes of both solutions in buffer. The dimensions of the observation volume were determined using a calibration sample with a known diffusion coefficient at 20 °C (Figure S11).

**Data Acquisition and Analysis for Correlation Spectroscopy Experiments.** The luminescence intensity was recorded in data sets of 40 s at a temporal resolution of 10 μs. Each data set was autocorrelated using an automated routine in Matlab. At least seven data sets were averaged per measurement and the experiment was repeated independently three times at each protein concentration. The autocorrelation functions G(τ) were analyzed in Matlab using a one species, three-dimensional diffusion model with an additional exponential term that accounts for nanorod rotation:

\[
G(\tau) = \frac{1}{(N)} \left(1 + \frac{\tau}{\tau_0}\right)^{-1} \left(1 + \frac{\tau}{\tau_0}\right)^{-2} \left(1 + \frac{\tau}{\tau_0}\right)^{-1.2} \left(1 + 4 \cdot e^{-(\tau_0/\tau)}\right)
\]

where \(\langle N\rangle\) denotes the average number of particles in the three-dimensional Gaussian-shaped observation volume, with radial and axial dimensions \(\tau_0\) and \(\tau_0\) respectively. The translational diffusion time \(\tau_0\) is related to the translational diffusion coefficient of the particles, \(D_0 = \gamma^2/(4R_0^2)\). An additional exponential term of amplitude \(A\) and rotational diffusion time \(\tau_{0r}\) was used to account for nanorotational diffusion. The translational diffusion coefficient was used to estimate the equivalent hydrodynamic radius of the nanoparticles using the Stokes–Einstein relationship

\[
R_0 = kT/6\pi\eta D_0
\]

Changes in viscosity due to the presence of BSA were calculated using the intrinsic viscosity of this protein (4.2 cm²/g) assuming a linear relationship in the range of concentrations used. Results of correlation spectroscopy experiments before and after BSA binding at different concentrations are shown in Table S1.
concentration was unknown, the solution was repeatedly diluted until single isolated nanowires were observed in the optical microscope. Circular Dichroism (CD) Spectroscopy. Ensemble CD measurements were taken on a J-815 circular dichroism spectrometer (JASCO) with a 1 mm path length quartz cuvette at 20 ± 0.1 °C using a Peltier temperature controller. Equal volumes of ~1 mM nanoparticle solutions (MUTAB-AuNRs or AuNPs) and 0.1 mg/mL BSA solutions were mixed ~30 min before each measurement. Wavelength ranges of 190–260 and 500–750 nm were collected every 1 nm under standard sensitivity, bandwidth of 1.00 nm, and rate of 50 nm/min. Four scans were collected for each trial and averaged. The spectra were baseline-corrected and presented as mean residue ellipticity. The solvent for CD measurements was 1 mM phosphate buffer (pH 7.2) due to its low absorbance in the far UV. This solvent did not affect the stability of MUTAB-AuNRs, MUTAB-AuNPs or BSA.

SEM Imaging. SEM images were taken using a FEI Quanta 400 ESEM FEG, operated at 10 kV under low vacuum conditions. Analysis of aggregate size was performed in ImageJ.

MUTAB-AuNR Uptake by MCF-7 Cells. Preincubation of MUTAB-AuNRs under Different Media Conditions before Incubation with MCF-7 Cells. For the experiments shown in Figure 1a,b, MUTAB-AuNRs were incubated in two different media conditions: (1) 1 mL of MUTAB-AuNRs was mixed with 133 μL of 7.5% (w/v) BSA (final concentration of BSA ~1% w/v) and incubated overnight. This formed a BSA-corona onto the MUTAB-AuNRs before exposure to serum. Next, the BSA-MUTAB-AuNRs were diluted (1:10) in EMEM with 10% FBS (with 1% PCN) and incubated overnight. (2) Then, 1 mL of MUTAB-AuNRs was directly diluted (1:10) in EMEM with 10% FBS (with 1% PCN) and incubated overnight. Both MUTAB-AuNR solutions were found to be stable in EMEM over the time scale of the experiments, as observed via UV/vis spectroscopy.

Nanoparticle Incubation with MCF-7 Cells. MCF-7 cells were seeded into two 6-well plates with each well containing 2 mL of 100 000 cells/mL solution and cultured for 3 days in EMEM with 10% FBS and 1% PCN (same as incubation conditions for MUTAB-AuNRs). On the third day, the media was removed and each well was carefully washed three times with PBS to remove adhered proteins. Two wells were filled with 2 mL media containing either 1% BSA, 10% FBS in EMEM with 1% PCN, or 10% FBS in EMEM with 1% PCN. The remainder of the wells contained 2 mL of the corresponding BSA-MUTAB-AuNR solutions, each in triplicate. The plates were incubated at 37 °C in 5% CO2 for 6 h. After incubation for 6 h, 50 μL of media from each well was removed in triplicate using a pipet and kept for the LDH assay. The wells were washed with PBS three times to remove adhered proteins and AuNPs. The cells were then detached by incubation in 0.05% trypsin with EDTA and counted using a standard hemacytometer (Figure S22). After counting, the cells were transferred to a solution of 0.1% Triton X-100 and placed in a −20 °C freezer overnight.

LDH Cytotoxicity Assay of MCF-7 Cells Incubated with BSA-MUTAB-AuNRs. A LDH assay was performed by adding 50 μL of formazan dye (Thermo Scientific) to each well containing media from the nanoparticle—cell incubation experiments (Figure S23). The plates were placed in an incubator at 37 °C with 4.5% CO2 for 30 min and read on a BioTek Uniread 800 plate reader. The purpose of this assay was to measure the cytotoxicity of the MUTAB-AuNRs under the different incubation conditions with BSA and FBS, compared to MCF-7 cells alone.

ICP-MS Measurement of BSA-MUTAB-AuNRs Uptaken by MCF-7 Cells. The lysed cells were thawed and centrifuged at 8000g for 10 min. The supernatant was removed and the pellets were digested using aqua regia (3:1 solution of HNO3 and HCl). Following digestion, the pellets were diluted to 10 mL using 5% HCl in milli-Q water. The samples were measured for Au concentration using a PerkinElmer Nexion 300 ICP-MS. The concentration of Au was determined for each cell using the cell count collected after detaching the cells (Figure S24).

Calculation of the Number of AuNRs Uptaken by Each Cell. The average number of AuNRs uptaken per cell was calculated by first determining the number of AuNRs in the different solutions with cells from the concentration of Au determined via ICP-MS. The number of AuNRs was then divided by the average number of cells. The addition of BSA to the MUTAB-AuNRs increases the uptake by MCF-7 cells by 320% compared to the conventional incubation conditions in EMEM with 10% FBS.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.5b06439.

Details regarding experimental and computational methods and data analysis (PDF)

Videos S1 and S2 (ZIP)

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Notes

The authors declare no competing financial interest.

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