Electronic Supplementary Information

Cellular binding of nanoparticles in the presence of serum proteins

Gerard W. Doorley and Christine K. Payne*

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

Cell Culture: BS-C-1 cells (ATCC, Manassas, VA, USA) were maintained in a 37^{o}C, 5% carbon dioxide environment in Minimum Essential Medium (MEM, Invitrogen, Carlsbad, CA, USA) with 10% (v/v) fetal bovine serum (FBS, Invitrogen). Cells were passaged every 3 days. For fluorescence imaging, cells were cultured in 35 mm glass-bottom cell culture dishes (MatTek, Ashland, MA, USA) and imaged in phenol red-free MEM (Invitrogen).

Nuclear Staining: The cell nucleus was stained with 55 μM 4',6-diamidino-2-phenylindole dilactate (DAPI, Invitrogen) at 37^{o}C for 1 hour.

Zeta Potential Measurements: The zeta potentials of the NPs in specified solutions were measured with a Malvern Zetasizer (Nano-ZS, Malvern Instruments, Worcestershire, UK) using a universal dip cell in disposable cuvettes. The Smoluchowski approximation was used to convert the electrophoretic mobility to a zeta potential. Experiments consisted of 30 runs per measurement and all experiments were carried out in triplicate. The mean of each triplicate measurement is shown. Error bars represent the standard deviation. The conductivity (mS/cm) of the samples varied linearly with increasing amounts of MEM or FBS in MEM. High concentrations of MEM (>10%) or FBS (>0.1%) in MEM resulted in a deviation from linearity. The measurements shown in Fig. 1 were made in general purpose mode with the exception of the 100% MEM solution. For this measurement data were collected in monomodal mode due to the high conductivity of this sample (14 mS/cm).

Microscopy and Image Analysis: Epi-fluorescence microscopy was carried out with an inverted microscope (Olympus IX71, Center Valley, PA, USA) with a 60x, 1.20 N.A., water immersion objective (Olympus). A xenon lamp was used for excitation and a CCD camera (DU-897, Andor, South Windsor, CT, USA) was used for detection. A FITC filter cube (Excitation filter: 480/40 nm, Chroma; Dichroic: FF506-Di02, Semrock; Emission filter: 536/40 nm, Semrock) was used to image the NPs and a Cy5 filter cube (Excitation filter: 620/60 nm, Chroma; Dichroic: FF660-Di01, Semrock; Emission filter: 692/40 nm, Semrock) was used to image the AF647-FBS or AF647-BSA. A DAPI filter cube (DAPI-1160A, Semrock) was used to image DAPI. Image J (http://rsb.info.nih.gov/ij/) was used for fluorescence image analysis and processing. The object-based analysis in JACo Plugin (http://rsbweb.nih.gov/ij/plugins/track/jacop.html, S. Bolte and F.P. Cordelieres, *J. Microscopy*, 2006, **224**, 213.) was used to measure colocalization. The threshold was adjusted manually for each set of images. Internalization was quantified by determining the percentage of NPs undergoing active transport, indicative of motor protein-dependent motion inside the cell. Images of FBS-NPs and BSA-NPs were recorded at a rate of 1 frame/s after an 18 hr incubation. Colocalization was measured for two frames separated by 15 s. For both FBS-NPs and BSA-NPs, colocalization was >95% over this time indicating a lack of active transport. In comparison, a particle such as low density lipoprotein that is readily internalized by the cell shows only 30%

Electronic Supplementary Information

colocalization after 15 s as the motion of the endocytic vesicles changes the position of the low density lipoprotein in the cell. The lack of internalization for the NPs is expected as they are relatively large (200 nm) and lack endocytic ligands. The brightness and contrast of the fluorescent images were increased for publication purposes. The individual images in ESI show the intensities of each channel set to equivalent values.

Supplementary Figures

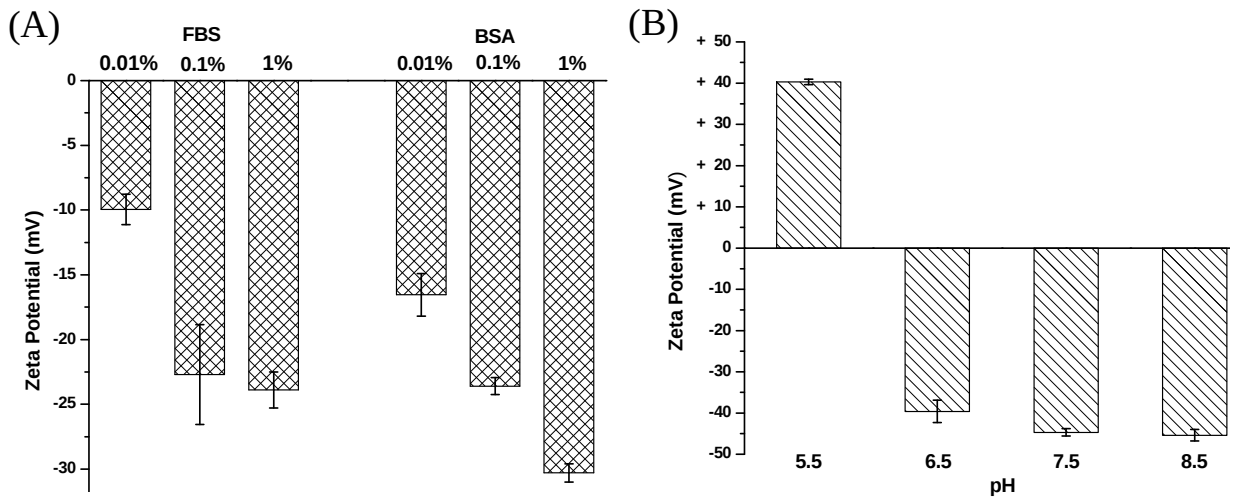


Fig. S1. (A) Zeta potential measurements of NPs and FBS or BSA in pure water show a negative effective surface charge in the presence of serum proteins. (B) Zeta potential measurements of NPs in water with increasing pH (adjusted using NaOH). Each pH value reflects a pH range of 1, i.e. pH 5.5 is a range of pH 5-6. While pure water is slightly acidic and MEM is slightly basic (pH 7.6), the zeta potential of NPs in MEM (Fig. 1) is not a reflection of pH, but is specific to MEM.

Electronic Supplementary Information

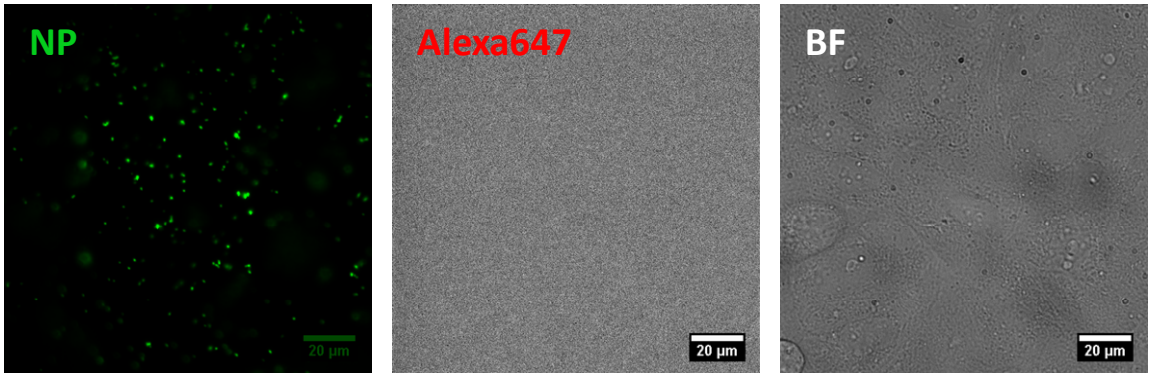
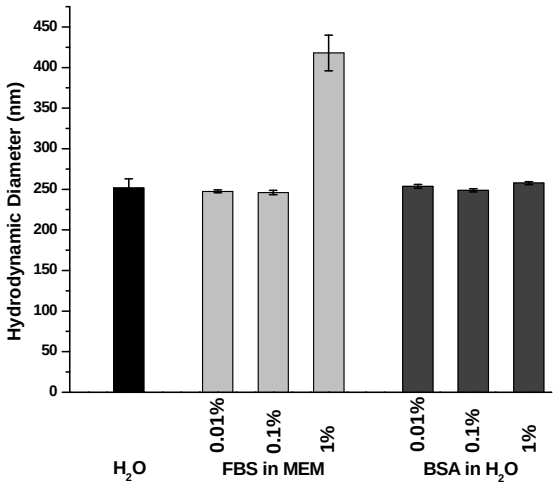


Fig. S2. The addition of excess hydroxylamine was used to stop the reaction of the AF647 with FBS or BSA and to prevent free AF647 from reacting with the amine-modified NPs and labeling them directly. As one control, a 30 mM hydroxylamine and 67 µM AF647 solution, in the absence of protein, was incubated with NPs for 1 hr at room temperature. This mixture was allowed to bind to cells for 10 minutes. The cells were then rinsed with phenol-red free MEM. The lack of AF647 signal shows that AF647 is rinsed out of the cell culture medium and does not label the NPs. BF is the brightfield image of the cells.



NPs	D_H (nm)	Std. dev. (nm)
H_2O	252	11
0.01% FBS in MEM	248	1.8
0.1% FBS in MEM	246	2.8
1% FBS in MEM	418	22
0.01% BSA in H_2O	254	2.5
0.1% BSA in H_2O	249	1.9
1% BSA in H_2O	258	1.8

Fig. S3. Low concentrations of FBS and BSA do not affect the hydrodynamic diameter of the NPs. At 1% FBS a significant size increase was observed, possibly due to aggregation. Cellular imaging experiment were carried out at 0.0036% FBS and 0.003% BSA. Based on the concentration of BSA and NPs, we calculate a maximum ratio of 8000 BSA molecules/NP. Based on previous work with citrate-coated gold NPs (S.H. Brewer, W.R. Glomm, M.C. Johnson, M.K. Knag, and S. Franzen, *Langmuir*, 2005, **21**, 9303.), we estimate a maximum surface coverage of 3.7 x 10^{12} BSA molecules/cm^2. While citrate-coated gold NPs differ from amine-modified polystyrene NPs, both NPs have an electrostatic interaction with the BSA allowing for a reasonable order of magnitude calculation. For 200 nm NPs, we estimate a monolayer consisting of 4800 BSA molecules. This value, which is 60% of our maximum number of BSA molecules/NP, along with the lack of increase in hydrodynamic diameter,

suggests that the BSA concentrations used for imaging experiments result in approximately a monolayer of BSA. Hydrodynamic diameters were measured with a Malvern Zetasizer (Nano-ZS, Malvern Instruments, Worcestershire, UK) using disposable cuvettes. Experiments were carried out in triplicate at a detection angle of 173°. The mean of each triplicate measurement is shown. Error bars represent the standard deviation. The table details the values plotted. FBS measurements were made in MEM supplemented with 10% FBS, diluted in water.

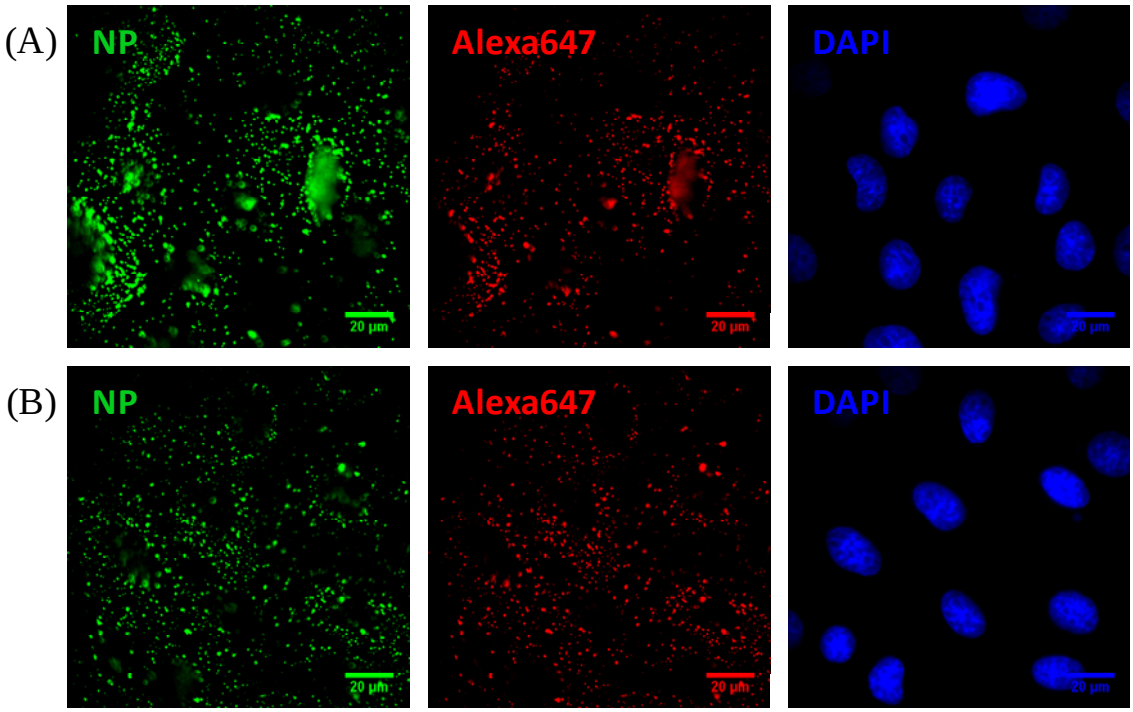


Fig. S4. Individual images used to construct the merged images shown in Figure 2. (A) FBS-NPs. (B) BSA-NPs. Intensities of each channel are set to equivalent values.

Electronic Supplementary Information

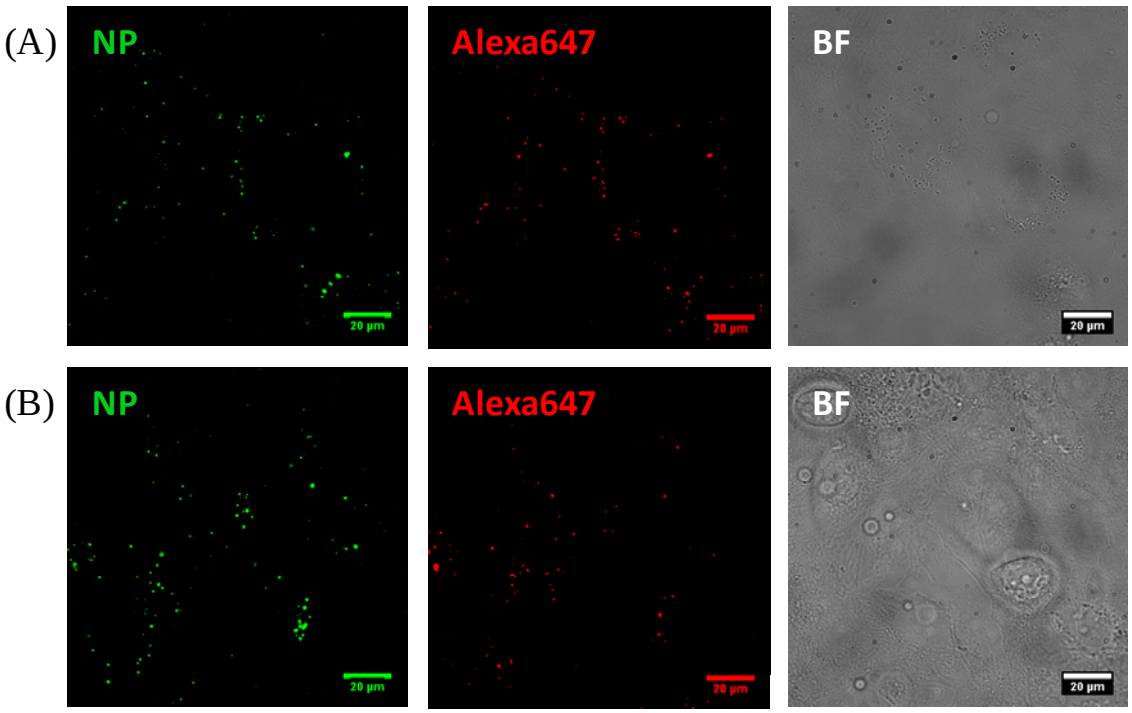
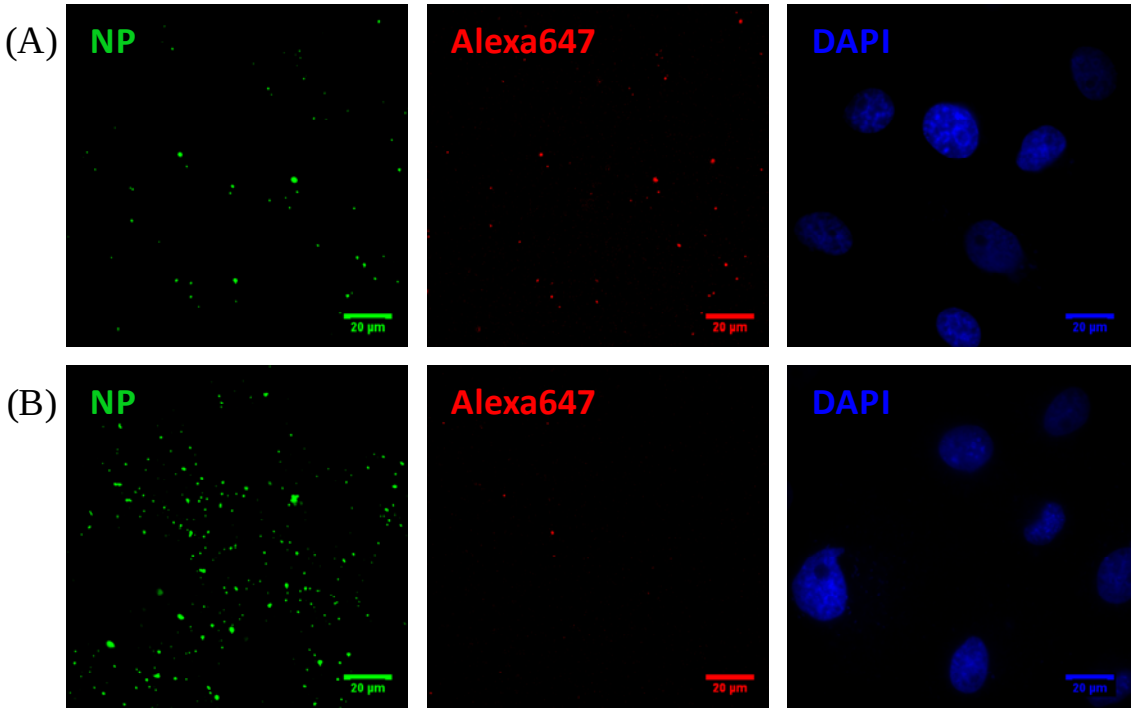


Fig. S5. Representative images of serum proteins and NPs after an 18 hr incubation in MEM with 10% FBS at 37°C. These and other images were analyzed to construct Fig. 3. (A) FBS-NPs. (B) BSA-NPs. Intensities of each channel are set to equivalent values. BF is the brightfield image of the cells.



Electronic Supplementary Information

Fig. S6. The limit of detection for fluorescence images was determined using decreasing concentrations of AF647-BSA and identical exposure times (150 ms). (A) At 0.1% of the concentration used for the imaging experiments described in the manuscript, the AF647-BSA signal (red) is visible and shows high colocalization with NPs (green). (B) At 0.01% it is not possible to measure colocalization. These images indicate that >99.9% of BSA molecules on the surface of an NP must be displaced before that NP is considered not colocalized. Nuclei are stained with DAPI (blue).

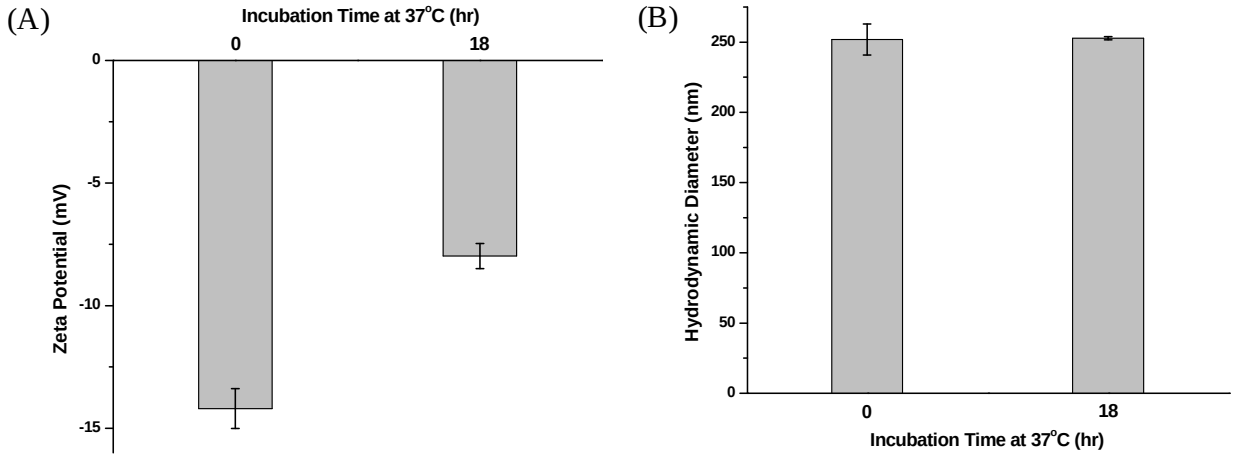


Fig. S7. (A) An 18 hr incubation of NPs with 0.01% FBS in MEM at 37°C leads to a slight increase in zeta potential, but the NP remains anionic. (B) Under the same conditions, no change in the hydrodynamic diameter of the NPs is observed.