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Membrane potential mediates the cellular binding of nanoparticles

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Abstract

The use of nanoparticles for cellular therapeutic or sensing applications requires nanoparticles to bind, or adhere, to the cell surface. While nanoparticle parameters such as size, shape, charge, and composition are important factors in cellular binding, the cell itself must also be considered. All cells have an electrical potential across the plasma membrane driven by an ion gradient. Under standard conditions the ion gradient will result in a -10 to -100 mV potential across the membrane with a net negative charge on the cytosolic face. Using a combination of flow cytometry and fluorescence microscopy experiments and dissipative particle dynamics simulations, we have found that a decrease in membrane potential leads to decreased cellular binding of anionic nanoparticles. The decreased cellular binding of anionic nanoparticles is a general phenomenon, independent of depolarization method, nanoparticle composition, and cell type. Increased membrane potential reverses this trend resulting in increased binding of anionic nanoparticles. The cellular binding of cationic nanoparticles is minimally affected by membrane potential due to the interaction of cationic nanoparticles with cell surface proteins. The influence of membrane potential on the cellular binding of nanoparticles is especially important when considering the use of nanoparticles in the treatment or detection of diseases, such as cancer, in which the membrane potential is decreased.

1 Introduction

The small size of nanoparticles (NPs) enables their interaction with cells, organelles, and even proteins making NPs uniquely suited for biological applications.^{1–9} A key step in many of these applications is the binding of the NP to the surface of a cell. Cellular binding of NPs depends on both the NP; its size, shape, charge, and composition, and the cell; its cell surface receptors and membrane composition.^{10, 11} Recent results have suggested that cellular membrane potential is an additional parameter that influences the interaction of NPs with cells.¹² All cells have an electrical potential across the plasma membrane driven by an ion gradient coupled with selective permeabilities.¹³ Under standard conditions the ion gradient will result in a -10 to -100 mV potential across the membrane with a net negative charge on the cytosolic membrane (Fig. 1). The cellular membrane potential is dominated by

a difference in cytosolic and extracellular potassium concentrations. Under physiological conditions, a greater concentration of K^+ exists in the cytosol than in the extracellular medium resulting in net efflux of K^+ out of the cell. K^+ is a relatively permeable ion that can passively cross the plasma membrane through K^+ channels. In comparison, negatively charged ions and proteins are less permeable and remain within the cytosol. Cells with a physiological membrane potential are referred to as “polarized.” When the membrane potential is decreased or removed, cells are “depolarized.” An increase in membrane potential results in “hyperpolarized” cells. The influence of membrane potential on NP binding is especially important when considering the use of NPs for the treatment or detection of diseases in which the membrane potential is altered. For example, the cellular membrane potential is significantly decreased in cancer cells,^{14, 15} defective neutrophils,^{16–18} and cells with disrupted ion channels.^{19, 20}

Our goal was to examine the cellular binding of negatively- and positively-charged NPs in response to depolarization and hyperpolarization of cells. Membrane potential was controlled by the insertion of exogenous ion channels or changes to extracellular ion concentrations. We focused on polystyrene NPs modified with either carboxylate or amine groups to provide an anionic or cationic NP surface, respectively. These model NPs allow for a comparison of NPs with the same composition, but opposite surface charges. We used two complementary experimental techniques, flow cytometry and fluorescence microscopy, to measure the cellular binding of NPs. For both types of experiments, cells were cooled to 4 °C to allow binding, but block endocytic uptake.^{21, 22} Experiments were carried out in two cell lines to ensure that results were not cell type-dependent. Experiments were complemented with dissipative particle dynamics simulations.

Experiments and simulations both show that the cellular binding of anionic NPs is significantly decreased following the depolarization of cells. In comparison, hyperpolarization of cells leads to increased cellular binding of anionic NPs. Flow cytometry experiments detected a slight increase in binding for cationic NPs in response to depolarization, confirmed with simulations. The relatively minor importance of membrane potential for cationic NPs, as compared to anionic NPs, is attributed to the use of membrane proteins as binding sites for cationic NPs. These studies demonstrate that cellular membrane potential is a parameter that must be considered in the design of NPs for cellular applications, especially the treatment or detection of disease states in which the membrane potential of cells is altered.

2 Experimental

2.1 Cell Culture

CHO cells (ATCC, Manassas, VA) were maintained in a 37 °C, 5% carbon dioxide environment in Ham's F-12 Medium (21700-075, Invitrogen, Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum (FBS, 10437028, Invitrogen). HeLa cells (ATCC) were maintained in a 37 °C, 5% carbon dioxide environment in Minimum Essential Medium (MEM, 61100061, Invitrogen) supplemented with 10% (v/v) FBS. Cells were passaged every 4 days. For fluorescence imaging, cells were cultured in 35 mm glass-bottom cell culture dishes (MatTek, Ashland, MA). Nuclei were stained with 27 μ M 4',6-diamidino-2-phenylindole diacetate (DAPI, D3571, Invitrogen) at 37 °C for 1 hour in MEM supplemented with 10% FBS.

2.2 Nanoparticles (NPs)

Fluorescent polystyrene NPs were used in the majority of cellular binding experiments. The diameter provided by the supplier is used to denote the NP: 40 nm carboxylate-modified (F8795, Invitrogen), 60 nm carboxylate-modified (FC02F/10027, Bangs Laboratories,

Fishers, IN), 93 nm carboxylate-modified (FC02F/8972, Bangs Laboratories), 190 nm (FC02F/9942, Bangs Laboratories), 87 nm amine-modified (C29029, Invitrogen), 100 nm amine-modified (L9904, Sigma Aldrich, Milwaukee, WI). Carboxylate-modified QDs (Q21341MP, Invitrogen) were used to ensure results were not specific to polystyrene NPs. The hydrodynamic diameters and zeta potentials of the NPs are provided in Table 1.

2.3 Dynamic light scattering (DLS) and zeta potential measurements

The hydrodynamic diameter and zeta potential of the NPs were measured with a Malvern Zetasizer (Nano-ZS, Malvern Instruments, Worcestershire, UK) in disposable cuvettes. Solutions of NPs in water were measured at the following concentrations: 40 nm carboxylate-modified (3.3 nM); 60 nm carboxylate-modified (700 pM); 93 nm carboxylate-modified (187 pM); 190 nm carboxylate-modified (21.9 pM); 87 nm amine-modified (173 pM), and 100 nm amine-modified (378 pM). Measurements were carried out in triplicate. Hydrodynamic diameter and zeta potential data was acquired from 30 runs per measurement and 20 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.

2.4 Depolarization of cells

Cells were depolarized using two methods. First, gramicidin A, a bacterial ion channel capable of inserting into the membrane of mammalian cells, was used to depolarize cells.^{13, 23} Its insertion into the membrane allows free passage of monovalent cations, such as sodium and potassium, thereby removing the ion gradient and depolarizing cells. Cells were pre-treated with gramicidin A (1 μ M in phosphate buffered saline (PBS)) for 10 min at 4 °C. The resulting decrease in membrane potential following gramicidin A treatment was measured using DiBAC (2 μ M, 10 min incubation, 4 °C, Invitrogen), a membrane potential sensitive bis-oxonol fluorophore.^{24–28} Control experiments in polarized cells were carried out in PBS containing calcium and magnesium. PBS lacking calcium and magnesium, supplemented with 1 μ M gramicidin A, was used for depolarization. The change in DiBAC signal in response to depolarization was monitored with flow cytometry (BD LSR-II) and fluorescence microscopy (Olympus IX71). As the membrane potential decreases, cells become more permeable to DiBAC resulting in an increased fluorescence intensity (Fig. S1). Similar levels of depolarization were obtained for CHO and HeLa cells. An analysis of cell health using a MTT assay showed that treatment with gramicidin A resulted in a 20% decrease in cell viability (Fig. S2). For flow cytometry and fluorescence microscopy experiments, cells were incubated with gramicidin A at 10 min at 4 °C. NPs were then added to the cells for an additional 10 min at 4 °C.

As a second method of depolarization, cells were incubated with PBS in which Na^+ salts are replaced with K^+ salts. The addition of extracellular K^+ decreases the K^+ flux across the membrane and has been shown previously to depolarize cells.^{29, 30} Our experiments used PBS with 40 mM Na^+ replaced with 40 mM K^+ , referred to as PBSK₄₀. As with gramicidin A, incubation with PBSK₄₀ led to depolarization of CHO cells as measured with DiBAC and flow cytometry (Fig. S3).

2.5 Hyperpolarization of cells

Cells were hyperpolarized by replacing the sodium chloride in HEPES buffer with choline chloride. Choline cations are unable to cross the plasma membrane, causing positive charge to accumulate on the exterior leaflet of the membrane.^{31–33} The hyperpolarizing media (ChoCl) consisted of 137 mM ChoCl, 5.4 mM KCl, 1.02 mM NaH_2PO_4 , 3.6 mM CaCl_2 , 0.8 mM MgSO_4 , and 10 mM HEPES in water (pH 7.4). HEPES was used for normally polarized cells. Cells were pre-treated with hyperpolarizing media for 10 minutes at room

temperature. The resulting increase in membrane potential was measured using DiBAC (2 μ M, 10 min incubation, 21 °C, Invitrogen) with flow cytometry (Fig. S4).

2.6 Flow cytometry

The CHO and HeLa cells used in these experiments are adherent cells that grow on the surface of cell culture flasks or dishes. Cells for flow cytometry were removed from the surface of cell culture flasks using Accutase (A6964, Sigma Aldrich). After removal from the cell culture flask, cells were pelleted (5000 rcf for 6 min) and resuspended in control, depolarization, or hyperpolarization media. The following concentrations of NPs were used for flow cytometry and imaging experiments: 40 nm carboxylate-modified (262 pM); 60 nm carboxylate-modified (56 pM); 93 nm carboxylate-modified (14.7 pM); 190 nm carboxylate-modified (1.73 pM); 87 nm amine-modified (17.1 pM); 100 nm amine-modified (15.1 pM), and carboxylate-modified QDs (320 pM). The cell suspensions were then filtered (0.4 μ m filter) for flow cytometry. Flow cytometry was carried out using a BD LSR-II flow cytometer (Becton Dickinson, Franklin Lakes, NJ). The polystyrene NPs are embedded with a yellow-green fluorescent dye and the FITC channel was used for detection. The QDs emit at 525 nm and the FITC channel was used for detection. For each experiment, ~15,000 cells in the population of interest were sampled. Populations for analysis were determined with a propidium iodide assay. Histograms were analyzed using Weasel 3.0.1 (Walter and Eliza Hall Institute of Medical Research, Victoria, Australia). Percent change of the mean intensity of the population was used to measure the change in NP binding. The percent change, rather than absolute change, takes into account differences in PMT voltages. A p-value of < 0.05 was used as a threshold to determine if changes in mean intensity were significant.

2.7 Fluorescence microscopy

Cells for imaging were prepared as described for flow cytometry and the same concentrations of NPs were used. As the cells are adherent, they remained on glass coverslips for the course of experiments and were rinsed with their respective medium immediately prior to imaging to remove unbound NPs. NPs bound to cells were imaged with an epifluorescence microscope (Olympus IX7, Center Valley, PA) using a 1.20 N.A., 60 $\times$, water immersion objective (Olympus). The NPs are embedded with a yellow-green fluorescent dye and a FITC filter cube (Excitation filter: 480/40 nm, Chroma, Bellows Falls, VT; Dichroic: FF506-Di02, Semrock, Rochester, NY; Emission filter: 536/40 nm, Semrock) was used for imaging NPs. A DAPI filter cube (DAPI-1160A, Semrock) was used to image DAPI. Emission was detected with an EMCCD (DU-897, Andor, South Windsor, CT). 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. The number of NPs per cell was quantified using Adjust Threshold and Analyze Particles. Brightness and contrast were set equally for all images for comparison.

2.8 Dissipative particle dynamics simulations

The use of dissipative particle dynamics to simulate NP-cell interactions has been described previously.³⁴ In brief, the motion of the particles, or beads, used to simulate molecules is determined by classical mechanics. Lipid molecules are constructed from three hydrophobic beads to form a headgroup and two hydrophobic tails, each tail consisting of five beads. This model of a lipid forms stable bilayers with expected phase behavior.^{35, 36} The NP is modeled as a spherical solid coated with ligands. Water molecules are modeled as single neutral beads. Counterions are represented as single beads with a single charge. Membrane tension was controlled by the lipid number per area.^{37,38}

3 Results and discussion

3.1 Characterization of NPs

Cellular binding of NPs as a function of membrane potential was measured using polystyrene NPs modified with either carboxylate (anionic) or amine (cationic) groups. NPs were characterized within our lab using a Zetasizer (Nano-ZS, Malvern Instruments) to confirm hydrodynamic diameter (d_h) and effective surface charge (ZP) (Table 1). NPs were diluted in water and measurements were carried out in triplicate. Throughout the text, the diameter provided by the manufacturer is used to refer to the NP. The hydrodynamic diameter is often larger than the diameter provided by the supplier, especially if the supplier-provided NP diameter was determined by TEM. For example, carboxylate-modified NPs with a d_h of 60 nm are described as 40 nm NPs and the 87 nm amine-modified NPs have a d_h of 153 nm. Carboxylate-modified semiconductor quantum dots (QDs) were used to ensure that results were not specific to polystyrene. A reliable d_h value could not be obtained for the QDs. Based on our raw data and literature reports, we estimate a d_h of approximately 10 nm.³⁹

3.2 Cellular binding of anionic NPs decreases following depolarization of cells

NP binding, independent of internalization, was measured using 4 °C cold-binding to allow binding, but block endocytic internalization of NPs.^{21, 22} The polystyrene NPs are embedded with a fluorophore and QDs are inherently photoluminescent allowing us to measure binding as a function of fluorescence intensity with both flow cytometry and fluorescence microscopy. For both types of experiments, cells were depolarized with gramicidin A (1 μ M, 10 min, Fig. S1 and S2). NPs were then added to the cells (10 min, 4 °C). A complete protocol is described in Experimental.

We first examined the binding of four anionic polystyrene NPs of increasing diameter (40 nm – 190 nm, carboxylate-modified) to Chinese hamster ovary (CHO) cells. Flow cytometry experiments were carried out in triplicate and mean fluorescence intensity was used to quantify NP binding, characterized as a percent change in binding following depolarization with gramicidin A. For all of these NPs, we observe that depolarization leads to fewer NPs bound to the cell surface (Table 2 and Fig. S5). The decrease in binding is size-dependent with larger NPs showing a larger decrease in cellular binding. It is worth noting that the two carboxylate-modified NPs with hydrodynamic diameters of 60 nm (40 nm, supplied by Invitrogen, and 60 nm, supplied by Bangs Laboratories) show the same 10% decrease in binding. It is possible that the greater decrease in binding of the larger NPs reflects the larger surface area of the NP interacting with the cell surface.

The decrease in cellular binding is highlighted for the 190 nm polystyrene NPs (Fig. 2 and Fig. S6). Flow cytometry shows a 33% decrease in binding following treatment of CHO cells with gramicidin A (Fig. 2a). Similarly, fluorescence microscopy shows NPs bound to untreated, polarized CHO cells (Fig. 2b) and gramicidin A-treated, depolarized CHO cells (Fig. 2c). Counting the number of NPs bound to each cell shows a 58% decrease in the average number of NPs per cell. This is a larger decrease than the 33% change observed with flow cytometry, likely due to a difference in the methods used.

We next used a different depolarization method to ensure that the decrease in binding was not unique to gramicidin A treatment. As a potassium ion gradient drives the membrane potential, cells can also be depolarized by the addition of extracellular potassium. PBSK₄₀, a solution in which 40 mM sodium is replaced with 40 mM potassium, leads to depolarization of CHO cells (Fig. S3). Flow cytometry was used to measure the cellular binding of 93 nm anionic NPs following depolarization with PBSK₄₀ (Fig. 3). Depolarization of CHO cells led to a 27% decrease in NP binding, slightly greater than the 13% decrease in binding

following depolarization with gramicidin A. The overall decrease in binding shows that the method of depolarization does not matter: treatment of cells with either gramicidin A or PBSK₄₀ leads to decreased cellular binding of anionic NPs. The greater percent change observed following depolarization with PBSK₄₀ is likely due to the greater extent of depolarization obtained with PBSK₄₀ (Fig. S3).

To determine if the decreased cellular binding of anionic NPs was unique to polystyrene NPs, we used flow cytometry to measure the cellular binding of semiconductor QDs with an anionic (ZP = -45 mV), carboxylate-modified surface. As with polystyrene NPs, QDs showed a decrease in cellular binding following depolarization of CHO cells (Fig. 4). Considering the estimated ~10 nm diameter of the QDs, the 25% decrease in cellular binding is larger than that expected for a polystyrene NP of similar diameter (<10%) suggesting that QDs may interact differently with the cell surface.

The experiments described above show that decreased membrane potential leads to decreased cellular binding of anionic NPs independent of depolarization method or NP composition. To ensure that decreased binding of anionic NPs was not cell type dependent, flow cytometry and imaging experiments were repeated using human cervical carcinoma (HeLa) cells. Unlike CHO cells, HeLa cells are cancer cells. Although cancer cells are generally less polarized than healthy cells,^{14, 15} the addition of gramicidin A further depolarizes these cells allowing for a comparison of NP binding (Fig. S1). We used flow cytometry and fluorescence microscopy to measure the cellular binding of 40 nm, 93 nm, and 190 nm, anionic, carboxylate-modified, polystyrene NPs. As with the CHO cells, depolarization of HeLa cells led to decreased binding of anionic NPs (Table S1 and Fig. S7 and Fig. S8).

Dissipative particle dynamics simulations were used to examine the interaction of anionic NPs with the plasma membrane and better understand the experimental results. The plasma membrane was modeled as a charged surface (38.76 nm × 38.76 nm) consisting of a mixture of cationic and neutral lipids. Depolarization with gramicidin A was modeled by a neutral membrane. In both cases, membrane tension was set to allow binding, but prevent endocytosis, equivalent to the experimental conditions. Anionic NPs were simulated as solid particles with a diameter of 7 nm and charge of -100e. Each simulation included 9 NPs. Binding was measured as the fraction of NPs that bound to the membrane after a 640 ns simulation. Results are averages of four independent simulations. A comparison of binding to a charged (polarized) and uncharged (depolarized) membrane shows a 40% decrease in binding (Fig. 5).

Flow cytometry, fluorescence microscopy, and dissipative particle dynamics simulations show that the cellular binding of anionic NPs decreases following depolarization of cells (Table 2, Figs. 2–5). This decrease in binding is general as it is independent of depolarization method, NP composition, or cell type. These observations demonstrate that the cellular binding of anionic NPs is influenced by membrane potential. It is likely that depolarization reduces the attractive interaction of the anionic NPs with the relatively positively charged extracellular face of the membrane. This is also observed in the size-dependent decrease observed for the cellular binding of polystyrene NPs. Larger NPs are more strongly affected by decreased membrane potential, possibly due to their larger surface area interacting with the plasma membrane.

3.3 Cellular binding of anionic NPs increases following hyperpolarization

If membrane potential is a driving force for the cellular binding of NPs, we expect that hyperpolarization, increasing the membrane potential, should lead to a reversal of the trend observed for depolarization. Cells were hyperpolarized by replacing sodium chloride in a

HEPES buffer with choline chloride, described in Experimental.^{31–33} The choline cation is too large to diffuse across the plasma membrane resulting in increased membrane potential (Fig. S4). HEPES buffer was used for measurements with polarized cells. The cellular binding of the 40 nm – 190 nm anionic, carboxylate-modified, polystyrene NPs was measured with flow cytometry in response to hyperpolarization (Table 2). As hyperpolarization is a more subtle effect than depolarization (Fig. S1 and Fig. S4), fluorescence microscopy, which is less sensitive than flow cytometry, was not used for hyperpolarization experiments. Additionally, we were unable to hyperpolarize HeLa cells, which may be a function of their cancer origin as cancer cells are typically depolarized relative to non-cancerous cells.^{14, 15}

Hyperpolarization resulted in increased cellular binding of anionic NPs with an increasing percent change for larger NPs, the exact opposite of the trend observed for depolarized cells. The magnitude of the percent change is less than depolarization, presumably because hyperpolarization is a smaller change to the cellular membrane. The increase in NP binding following hyperpolarization of CHO cells is illustrated for the 190 nm polystyrene NPs (Fig. 6). Hyperpolarization of CHO cells leads to a 15% increase in NP binding as compared to a 33% decrease in binding following depolarization.

3.4 Cellular binding of cationic NPs is minimally affected by depolarization

We next measured the cellular binding of cationic, amine-modified, polystyrene NPs in response to depolarization of CHO cells. Flow cytometry experiments with cationic 87 nm NPs showed no change in cellular binding following depolarization of CHO cells with gramicidin A. The cationic 100 nm NPs showed a slight (10%) increase (Table 2). Unlike the anionic NPs, the cationic NPs with a smaller hydrodynamic diameter (100 nm, $d_h=105$ nm) had the larger change in binding following depolarization. HeLa cells showed a similarly slight increase in binding (+9% for 87 nm NPs) following depolarization (Table S1). Fluorescence microscopy of both cell lines showed no clear change in binding following depolarization. Considering the small changes observed following depolarization, hyperpolarization, which is a smaller perturbation to the system, was not investigated. Compared to experiments, dissipative particle dynamics simulations showed a clear increase in the cellular binding of cationic NPs (7 nm, +100e) following depolarization (Fig. 7).

As the simulations of cationic NPs showed a much greater increase in binding than the experimental results, we considered additional experimental factors that could influence the cellular binding of cationic NPs. Cationic NPs are known to bind to anionic proteoglycans and surface proteins.^{40–42} Surface proteins were incorporated into the simulations with the addition of a 6×6 array of negatively charged proteins (Fig. 8). Incorporating these proteins into the simulation resulted in 81% of the cationic NPs bound to the charged membrane with an increase to 97% following depolarization. It is likely that this slight increase in NP binding would not be resolved using our experimental methods. As depolarization does not affect the membrane proteins, it is reasonable that binding of cationic NPs is minimally affected by changes to the membrane potential. As expected, the addition of anionic proteins to the simulations reduced the overall binding of the anionic NPs, but decreased binding (–80%) following depolarization was still observed (Fig. S9).

Previous studies using instrumental neutron activation analysis showed that the cellular uptake of cationic gold NPs (2 nm core, 9 nm with ligand, +24 mV) decreased following depolarization of cells.¹² In comparison, we observe a very small increase in the cellular binding of cationic polystyrene NPs in response to depolarization. It is possible that the difference between the previous study and ours is due to the measurement of NP internalization as compared to NP binding or differences in NP and ligand composition. The previous studies also showed that cellular depolarization led to an increased calcium

concentration, which can affect cellular signaling and health. It is possible that cellular binding of the gold NPs is unchanged or slightly increased, but internalization is decreased due to a change in calcium levels. As a point of comparison, the cellular internalization of polio virus is decreased following both depolarization and hyperpolarization suggesting that changes to the membrane potential can have broad effects on cellular uptake.⁴³

4 Conclusion

Cellular binding of NPs is the essential first step for applications ranging from cellular imaging to the treatment of human disease. Using flow cytometry, fluorescence microscopy, and dissipative dynamics simulations, we have shown that the cellular binding of anionic NPs is decreased following cellular depolarization (Table 2, Figs. 2 – 5). The decreased cellular binding of anionic NPs was independent of depolarization method (Fig. 3), NP composition (Fig. 4), and cell type (Table S1). Hyperpolarization led to a reversal of this trend with increased cellular binding of anionic NPs (Fig. 6). In comparison, cationic NPs were minimally affected by a decrease in membrane potential (Table 2), possibly due to a greater interaction with cell surface proteins rather than directly with the plasma membrane (Fig. 7 and Fig. 8). These experiments show that membrane potential is an important factor that must be considered in the design of NPs for therapeutic and sensing applications. This is especially important considering that decreased membrane potential is a signature of cancer cells.^{14, 15} As nanomedicine applications move forward, it will be important to consider how decreased membrane potential will affect the cellular binding of NPs used to treat or detect cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

The ion gradient across the plasma membrane results in a membrane potential with a net negative charge on the cytosolic membrane. The membrane potential can be decreased, resulting in the depolarization of cells, using the exogenous ion channel gramicidin A. The membrane potential can also be increased, resulting in the hyperpolarization of cells, by replacing sodium ions with non-diffusible choline ions.

Fig. 2. Cellular binding of anionic NPs is decreased following depolarization. (a) Flow cytometry shows a decrease in the binding of 190 nm, carboxylate-modified, polystyrene NPs to CHO cells. Experiments were carried out in triplicate and error bars show standard deviation. (b) Fluorescence microscopy image of 190 nm NPs bound to CHO cells. (c) Depolarized CHO cells show reduced binding of NPs. The raw flow cytometry data and individual images used to construct Figs. 2b and c are included in ESI (Fig. S6). Similar results were obtained for the 40 nm, 60 nm, and 93 nm NPs (Table 2 and Fig. S5) as well as HeLa cells (Table S1 and Figs. S7 and S8). P-values < 0.01 are indicated by **.

Fig. 3. The use of extracellular potassium (PBSK₄₀), rather than gramicidin A, to depolarize cells results in decreased cellular binding of anionic NPs. Flow cytometry results are shown for 93 nm, carboxylate-modified, polystyrene NPs bound to CHO cells. Experiments were carried out in triplicate and error bars show standard deviation. P-values < 0.05 are indicated by *.

Fig. 4. The cellular binding of anionic, carboxylate-modified, QDs is decreased following the depolarization of CHO cells with gramicidin A. Flow cytometry experiments were carried out in triplicate and error bars show standard deviation. P-values < 0.001 are indicated by ***.

Fig. 5. Dissipative particle dynamics simulations show decreased cellular binding of anionic NPs to a neutral membrane, similar to the effect of gramicidin A. (a) Snapshot from a simulation showing anionic NPs and a charged membrane. The NP core is shown in green, anionic ligands in gray, cationic lipids in yellow, and neutral lipids in red. Counterions and water are not shown. (b) A snapshot from a simulation of NPs and a neutral membrane shows the reduced binding of anionic NPs. (c) Binding is measured as the fraction of NPs bound to the membrane after a 640 ns simulation.

Fig. 6. The hyperpolarization of CHO cells with choline chloride (ChoCl) leads to increased cellular binding of anionic, 190 nm, carboxylate-modified, polystyrene NPs. Flow cytometry experiments were carried out in triplicate and error bars show standard deviation. P-values < 0.05 are indicated by *. Increased binding following hyperpolarization was observed for all of the anionic NPs tested (Table 2).

Fig. 7. Dissipative particle dynamics simulations show increased cellular binding of cationic NPs to a neutral membrane. (a) Snapshot from a simulation showing cationic NPs and a charged membrane. The NP core is shown in green, cationic ligands in pink, cationic lipids in yellow, and neutral lipids in red. Counterions and water are not shown. (b) A snapshot from a simulation of NPs and a neutral membrane shows the increased binding of cationic NPs. (c) Binding is measured as the fraction of NPs bound to the membrane after a 640 ns simulation.

Fig. 8.

Dissipative particle dynamics simulations of a membrane, including anionic proteins, show slightly increased cellular binding of cationic NPs to a neutral membrane. (a) Snapshot from a simulation showing cationic NPs and a charged membrane. The NP core is shown in green, cationic ligands in pink, cationic lipids in yellow, and neutral lipids in red. Anionic proteins are shown in black. Counterions and water are not shown. (b) A snapshot from a simulation of NPs and a neutral membrane with anionic proteins shows the slightly increased binding of cationic NPs. (c) Binding is measured as the fraction of NPs bound to the membrane after a 640 ns simulation.

Table 1

Hydrodynamic diameter (d_h) and zeta potential (ZP) of the polystyrene NPs used in the course of experiments.

NPs	d_h (nm) ^a	ZP (mV) ^a
<i>40 nm COOH</i>	60 ± 0.9	-43 ± 2
<i>60 nm COOH</i>	60 ± 0.7	-36 ± 1
<i>93 nm COOH</i>	99 ± 0.4	-32 ± 0.7
<i>190 nm COOH</i>	191 ± 2	-35 ± 0.4
<i>87 nm NH₂</i>	153 ± 3	39 ± 4
<i>100 nm NH₂</i>	105 ± 1	28 ± 7

^aExperiments were carried out in triplicate. Error shows standard deviation.

Table 2

Summary of flow cytometry results for the binding of anionic and cationic NPs to CHO cells. Results for HeLa cells, which were similar, are shown in Table S1.

Charge	Size	Depolarized				Hyperpolarized			
		PBS Intensity	Gramicidin A Intensity	% Change	HEPES Intensity	ChoCl Intensity	% Change		
Anionic	40 nm	116.7 ± 3.0	105.1 ± 4.2	- 10 ± 4	117.4 ± 0.2	122.6 ± 2.3	+ 4 ± 2		
	60 nm	35.5 ± 0.2	32.0 ± 0.6	- 10 ± 2	32.6 ± 0.6	33.9 ± 0.5	+ 4 ± 2		
	93 nm	80.3 ± 3.7	69.9 ± 2.8	- 13 ± 6	29.4 ± 0.4	31.0 ± 0.6	+ 5 ± 2		
	190 nm	24.4 ± 1.7	16.3 ± 0.9	- 33 ± 8	31.1 ± 1.9	36.3 ± 1.8	+ 17 ± 8		
Cationic	87 nm	24.5 ± 0.8	24.6 ± 1.3	+ 0.4 ± 6	-	-	-		
	100 nm	66.4 ± 3.0	73.2 ± 1.5	+ 10 ± 5	-	-	-		