Targeted Cytosolic Delivery of Cell-Impermeable Compounds by Nanoparticle-Mediated, Light-Triggered Endosome Disruption

Supporting Information

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Materials and Methods

Nanoparticles synthesis, purification and functionalization

Before synthesis, all glassware was washed overnight in a base bath and thoroughly rinsed using ultrapure water. In a typical synthesis, 200 mg of cetyltrimethylammonium bromide (CTAB, Sigma) and 250 mg of Pluronic® F-127 (Sigma) were placed in an Erlenmeyer flask and dissolved in 100 mL of ultrapure water. To this solution, 0.7 mL of 2 M NaOH were added and the mixture was placed in a water bath at 80º C and magnetically stirred at 400 rpm. In a polypropylene tube (Falcon), a mixture of 1 mL of tetraethyl ortho-silicate (TEOS, Sigma) and 0.2 mL of mercaptopropyl trimethoxysilane (MPTMS, Sigma) was freshly prepared and rapidly injected into the stirring reaction mixture. The solution turned from clear to slightly opaque within a few minutes, indicating the formation of nanoparticles (NPs), and stabilized after ~20 min. The reaction was allowed to continue for 2 h at 80º C, cooled to room temperature, and filtered at 0.2 μm (Nalgene).

Extraction of the CTAB templating surfactant from the pores was performed by adding to the filtered particles an equal volume of ethanol, followed by hydrochloric acid (36% w/w) up to a final concentration of 0.1 M. The resulting mixture was magnetically stirred at 60ºC overnight. The NP suspension was then transferred to a dialysis cassette (Slide-A-Lyzer 7,000 MWCO, Pierce Biotechnology) and dialyzed against 50% ethanol/water at 40ºC overnight, then against purified water (18 MΩ) for another 12 h. Removal of the secondary surfactant, Pluronic® F-127, was performed by repeated concentration and dilution cycles in 50% ethanol using Amicon® Ultra-15 centrifugal filter units (Millipore). This step was critical, as extra washing cycles performed after partial removal of Pluronic® F-127 would induce nanoparticle aggregation. We speculate that the optimal number of

washing cycles removes enough Pluronic® F-127 to allow access to thiol groups for streptavidin attachment, but does not completely remove the protective layer, thus avoiding NP aggregation during the following conjugation steps.

After purification from templating and secondary surfactants, NPs were functionalized with streptavidin by covalent attachment via a hetero-bifunctional crosslinker with a 5 kDa poly-ethylene-glycol (PEG) spacer arm (NHS-PEG-maleimide, JenKem), or with shorter arm crosslinkers such as LC-SMCC (Pierce Biotechnology). Streptavidin (5 mg/mL) was typically first reacted with the crosslinker in phosphate buffered saline (PBS) at a molar ratio of approximately 10:1 crosslinker molecules per streptavidin for 10 min, then transferred into the NP solution (100 μg SA/ 2 mg NPs), and reacted overnight at 4º C. To protect the particles from aggregation in physiological buffers, 5 kDa PEG-maleimide (Rapp Polymere, Germany) freshly dissolved in water was added to the nanoparticles solution to a final concentration of 4 mg/mL, and incubated for >2 h at 4º C. Finally, PBS 10X concentrate was added to the NP suspension to reach physiological osmolarity (300 mOsm). Unbound streptavidin was removed by size-exclusion chromatography (Sephacryl® S-400 column, GE Healthcare). At this point, particles could be loaded with the desired cargo molecule. Typically, NPs were loaded with Alexa546-maleimide (Invitrogen) at 50 μM by overnight incubation, resulting in both covalently immobilized dye as well as hydrolyzed dye adsorbed non-specifically onto the silica surface and within the pores. A second round of size-exclusion chromatography removed unbound Alexa546.

For preparation of the PEI-PEG coated particles, the surfactant washed NPs (2mg/mL) were incubated with 25 ug/mL of 25 kDa polyethyleneimine (Sigma, titrated to pH 7), followed by addition of 5kDa PEG-maleimide (3mg/mL) as detailed above. Dye loading and washing steps were performed as above.

Synthesis of gold-core/mesoporous-silica-shell nanoparticles

Gold nanoparticles were synthesized using the standard Turkevitch method[1]. All glassware was washed in a basic solution (NaOH/Ethanol) or with *aqua regia* (2/3 HCl, 1/3 HNO_3). 200 mL of a 0.25 mM solution of $HAuCl_4$ was boiled while magnetically stirring at ~ 440 rpm. To this solution, 3.4 mL of a 50 mM aqueous solution of trisodium citrate was added to obtain nanoparticles of ~15 nm diameter (determined by TEM) and an estimated concentration of ~2 nM. This nanoparticle solution was then used in the coating reaction.

200 mg of CTAB and 500 mg of Pluronic® F68 (Sigma) were dissolved in 100 mL of gold nanoparticles. 0.7 mL of 2 M NaOH was added to the mixture, which was then placed in a water bath at 80º C under magnetic stirring at 400 rpm. In a separate tube, a mixture of 1 mL of TEOS and 0.2 mL of MPTMS was freshly prepared and rapidly injected into the gold nanoparticle mixture under vigorous stirring. The solution turned from clear red to slightly opaque within a few minutes indicating the formation of nanoparticles, and stabilized after approximately 20 min. The reaction was allowed to continue for 2 h at 80º C. The mixture was then allowed to cool to room temperature and was filtered at 0.2 μm (Nalgene).

Synthesis of gold-nanorod-core/mesoporous-silica-shell nanoparticles

Gold nanorods (NR) were synthesized following the method developed by Jana and Murphy[2]. Briefly, 14.5 g of CTAB were dissolved in 200 mL of ultrapure water (18 MΩ) and kept at 37° C with magnetic stirring at 120 rpm. A 5 mL aliquot was taken from this solution for the preparation of seeds. To the CTAB solution, 6 mL of 4 mM $AgNO_3$ aqueous solution was added, followed by 200 mL of 1 mM $HAuCl_4$ aqueous solution, and by 2.25 mL of a 0.1 M ascorbic acid solution.

Preparation of the gold seeds: 5 mL of 0.5 mM $HAuCl_4$ (Sigma) was added to 5 mL of the original

CTAB solution, followed (under continuous stirring) by 0.6 mL of ice-cold 10 mM $NaBH_4$ (Sigma). To induce formation of gold nanorods, 400 μL of gold seeds were then added to the growth solution while stirring at 200 rpm.

Mesoporous silica coating procedure: 40 mL of the previously prepared gold NR solution were washed X3 by centrifugation, and re-suspended each time in a 2 mg/mL solution of CTAB in water, the final CTAB concentration used in the coating reaction. The washed NRs were mixed with 80 mL of a 2 mg/mL CTAB solution in ultrapure water, to which 440 mg of Pluronic® F68 was added. After addition of 0.7 mL of 2 M NaOH, the mixture was heated to 80° C, and the pre-mixed silicate precursors (1 mL TEOS + 0.2 mL MPTMS) were rapidly injected. The resulting particles were allowed to cool to room temperature, passed through a 200 nm filter, and CTAB extracted in a 1% HCl in 50% ethanol/water solution overnight, followed by several washes in 50% ethanol/water. The large amount of silicate precursors used in the synthesis relative to the amount of nanorods results in the formation of multiple small pure silica nanoparticles in addition to coated NRs. However, these additional particles are easily removed after a few rounds of centrifugation/resuspension (*Supplementary Fig. S3*)).

Synthesis of magnetic-core/mesoporous-silica-shell nanoparticles

Mixed magnetite/maghemite nanoparticles were obtained via aqueous synthesis following the Massart process[3]. Briefly, 10 mL of 1 M $FeCl_3$ in water and 2.5 mL of 2 M $FeCl_2$ in 2 M HCl were added to 125 mL of a 0.7 M ammonia aqueous solution under magnetic stirring. The reaction was allowed to proceed for 2 h, after which the brown precipitate was magnetically decanted, washed with deionized water, and peptized in 1 M tetramethylammonium hydroxide (125 mL).

Mesoporous silica coating procedure: 20 mL of the previously synthesized magnetic cores were

centrifuged at 14,000 rpm for 1 h on a tabletop centrifuge and resuspended in 20 mL of a 14 mM tetramethylammonium hydroxide solution. In order to eliminate aggregated particles, the resuspended NPs were centrifuged again at 10,000 rpm for 20 min, and the supernatant was collected. 20 mL of magnetic cores were slowly introduced into an Erlenmeyer flask containing 80 mL of a 14 mM water solution of tetramethylammonium hydroxide (replacing NaOH in the mixture), 200 mg of CTAB, and 500 mg of Pluronic® F68. The mixture was heated to 80° C, after which 1 mL of TEOS premixed with 0.2 mL of MPTMS was rapidly injected. The reaction was allowed to proceed at 80° C for 2 h. After cooling to room temperature, the NPs were filtered at 0.2um. Surfactant extraction was carried out as described for other particle types.

Varying the amount of magnetic cores introduced in the synthesis, as well as the amount of secondary surfactant, allowed formation of NPs of varying diameter (*Supplementary Fig. S3*).

Synthesis of quantum-dot-core/mesoporous-silica-shell nanoparticles

Fort Orange quantum dots (QD) with emission at 600 nm were purchased from Evident as a 10 mg/mL toluene solution. In a typical reaction, 100 μL of the QD solution (0.1 mg) was transferred to methanol by addition of 400 μL of 3:1 methanol: isopropanol mixture followed by centrifugation, drying and sonication into 200 μL of pure methanol.

200 μL QDs in $CHCl_3$ were added to 4 mL of a 10 mg/mL CTAB solution in water under stirring and heated to 60-70^{o} C to evaporate the methanol from the microemulsion. 2 mL (0.05 mg QDs) of the resulting solution were added to 1.5 mL of a 20 mg/mL solution of Pluronic® F127 in water, 6.5 mL water and 70 μL of 2 M NaOH. The mixture was sonicated and centrifuged for 30 min at 12,000 rpm to remove QD-surfactant aggregates, heated to 80^{o} C, and 100 μL TEOS were added. After 30 min, another 70 μL NaOH were added, followed by 100 μL TEOS and 20 μL MPTMS. The reaction was

allowed to proceed for an additional 2 h, after which the NPs were allowed to cool to room temperature and filtered at 0.2 μm.

Dynamic light scattering size and zeta potential measurements

Measurements were performed on dilute suspensions in ultrapure water after pH neutralization using a Zetasizer Nano ZS instrument (Malvern). For functionalized NPs, the measurements were performed on dilute suspensions of NPs in PBS.

Loading and characterization of fluorescent compounds into mesoporous silica nanoparticles.

NPs were obtained at 2.75 mg/mL Pluronic® F-127 in the reaction mixture. Particles dissolved at 60 μg/mL were incubated overnight with the dye, after which the unbound dye was removed by centrifugation and re-suspension. The amount of immobilized dye was calculated by subtracting the supernatant fluorescence from the total amount initially added.

Construction of P-glycoprotein-GFP cDNA and the stable cell line

The hMDR1 (ABCB1) coding sequence was amplified by PCR from the *pHaMDRwt* plasmid (mammalian retroviral expression vector deposited by M. Gottesman in the Addgene database; Addgene plasmid N. 10957) using the forward primer: 5'- TAGCCACCATGGATCTTGAAGGGGAC -3' and the reverse primer: 5'- CCTTACCGGTTCCACTTCCCTGGCGCTTTGTTCCAG -3'. The PCR product was digested with NheI and AgeI, and sub-cloned into the NheI and XmaI restriction sites of the pEGFP-N2 mammalian expression vector (Clontech).

LN-229 cells (ATCC) were transfected (Lipofectamine 2000, Invitrogen) with the P-Glycoprotein-GFP construct and propagated in DMEM supplemented with penicillin/streptomycin, 10% fetal bovine serum, and 1 mg/mL neomycin to select for stably expressing cells. Selection of the MDR phenotype

was performed by incubation with the mitochondrial marker tetramethylrhodamine esther (TMRE, Sigma), a known substrate of P-glycoprotein, for 20 min (50 nM in complete medium), followed by FACS sorting (FACSCalibur, BD) to select GFP-positive and TMRE-negative populations. After this round of selection (which produced high- and low-P-gp-GFP-expressing cells), the population was propagated in the same medium, supplemented with 0.5 mg/mL neomycin.

Vesicle characterization

For experiments involving the Rab-5, Rab-7 and Lamp-1 markers, LN-229 cells (ATCC) were transfected with the corresponding GFP fusion construct using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions and propagated in DMEM supplemented with penicillin/streptomycin, 10% fetal bovine serum. One day post-transfection, cells were trypsinized and re-plated on glass coverslips. After 24 hours, the coverslips were washed X3 in D-PBS, and incubated with 20ug/mL PEI-PEG NPs in D-PBS for 30 min at room temperature. The coverslips were then washed X3 in D-PBS to remove unbound excess NPs, and incubated at 37º C with 5% CO_2 in DMEM supplemented with penicillin/streptomycin. The cells were taken out of the incubator at various time points, and quickly washed X3 in D-PBS before confocal imaging at room temperature (FluoView-1000, Olympus).

For LysoTracker staining, following NP uptake, the cells were incubated with 50 nM LysoTracker Blue DND-22 (Invitrogen) at 37º C with 5% CO_2 in DMEM supplemented with penicillin/streptomycin. After 30 min of incubation, the coverslips were washed X3 in D-PBS before confocal imaging at room temperature.

P-glycoprotein targeting and light-activated release

In a typical targeting experiment, cells were trypsinized, plated onto glass coverslips and grown to

~70% confluence. After removal from the incubator, coverslips were transferred to a Dulbecco-modified phosphate buffered saline (D-PBS, Gibco-Invitrogen) solution supplemented with 10 mM glucose and kept at room temperature for the duration of the experiment. Coverslips were first stained with mouse-anti-human-P-gp primary antibody (BD) at 2.5 μg/mL in the presence of 1% bovine serum albumin (BSA), followed by a biotinylated goat-anti-mouse secondary antibody (Invitrogen) at 5 μg/mL. Streptavidin-functionalized nanoparticles were diluted at 20-100 μg/mL in D-PBS and applied to antibody stained coverslips for 20-30 min, after which they were transferred back to growing medium and incubated at 37º C to allow endocytosis. After NP uptake, coverslips were transferred to a live imaging chamber in D-PBS and observed by laser scanning fluorescence confocal microscopy (FluoView-1000, Olympus). To induce cytosolic release of Alexa546, cells were exposed to green excitation light from a mercury arc lamp through a TRITC filter and focused by a 60X water-immersion objective onto the field of view, for durations ranging from 3 s to 120 s. Light fluence over the 200 μm x 200 μm field of view was measured through a mask with an equally sized pinhole and found to be 500 mW/cm^2 (ThorLabs PM100D optical power meter, S130VC probe).

Dextran, NeutrAvidin, and QD release experiments

LN-229 cells were biotinylated at room temperature with NHS-PEO$_4$-biotin in D-PBS (500 μM, Pierce) supplemented with 10 mM glucose for 30 min, washed X5 in D-PBS, and trypsinized in 0.01% trypsin/EDTA. Cells were collected with DMEM-10% FBS, centrifuged for 5 min at 1,000 rpm, and resuspended in a 50 μg/mL solution of dye-loaded NP-streptavidin in D-PBS-glucose. After 30 min, the cells were washed twice in D-PBS, resuspended in DMEM-10% FBS, and plated on 12 mm glass coverslips at a density of ~10^5 cells/cm^2. Dextran-FITC (3 kDa, Sigma), NeutrAvidin-FITC (Pierce), or QD525-streptavidin conjugates (Invitrogen) were added to the cells at respective concentrations of 1 mg/mL, 5 μg/mL, and 2 nM. Cells were placed in a 37° C incubator overnight with 5% CO_2 to allow

for coverslip attachment and simultaneous endocytosis of the surface-bound NP-streptavidin conjugates, as well as dextran, NeutrAvidin, or QDs present in the culture medium. After overnight endocytosis, cells were washed twice in D-PBS, and transferred to a live-cell imaging chamber for light stimulation and observation in D-PBS supplemented with 10 mM glucose. Cells were exposed to light from a mercury arc lamp through a TRITC filter as described in Methods, for durations varying from 30 to 120 s, and subsequent changes in the fluorescence distribution within the cells were observed for 30 min.

For experiments involving two types of NPs, the orange pseudo-colored NPs were the post-synthesis Alexa546 loaded NPs used in all other experiments, while the red pseudo-colored NPs were covalently bound to Alexa633 incorporated during synthesis of the NPs (1 μL of MPTMS and 20 μL of a 10 mM stock solution of Alexa633-maleimide were incubated overnight at room temperature, and premixed with the silicate precursors before injection into the reaction mixture. Unreacted dye was removed during the surfactant extraction process). The "orange" nanoparticles can release part of their cargo, while the dye is irreversibly bound to the silica matrix for the "red" NPs. After trypsinization, the biotinylated LN-229 cells were incubated separately with each type of NP, and remixed at 1:1 before plating and overnight incubation with dextran-FITC.

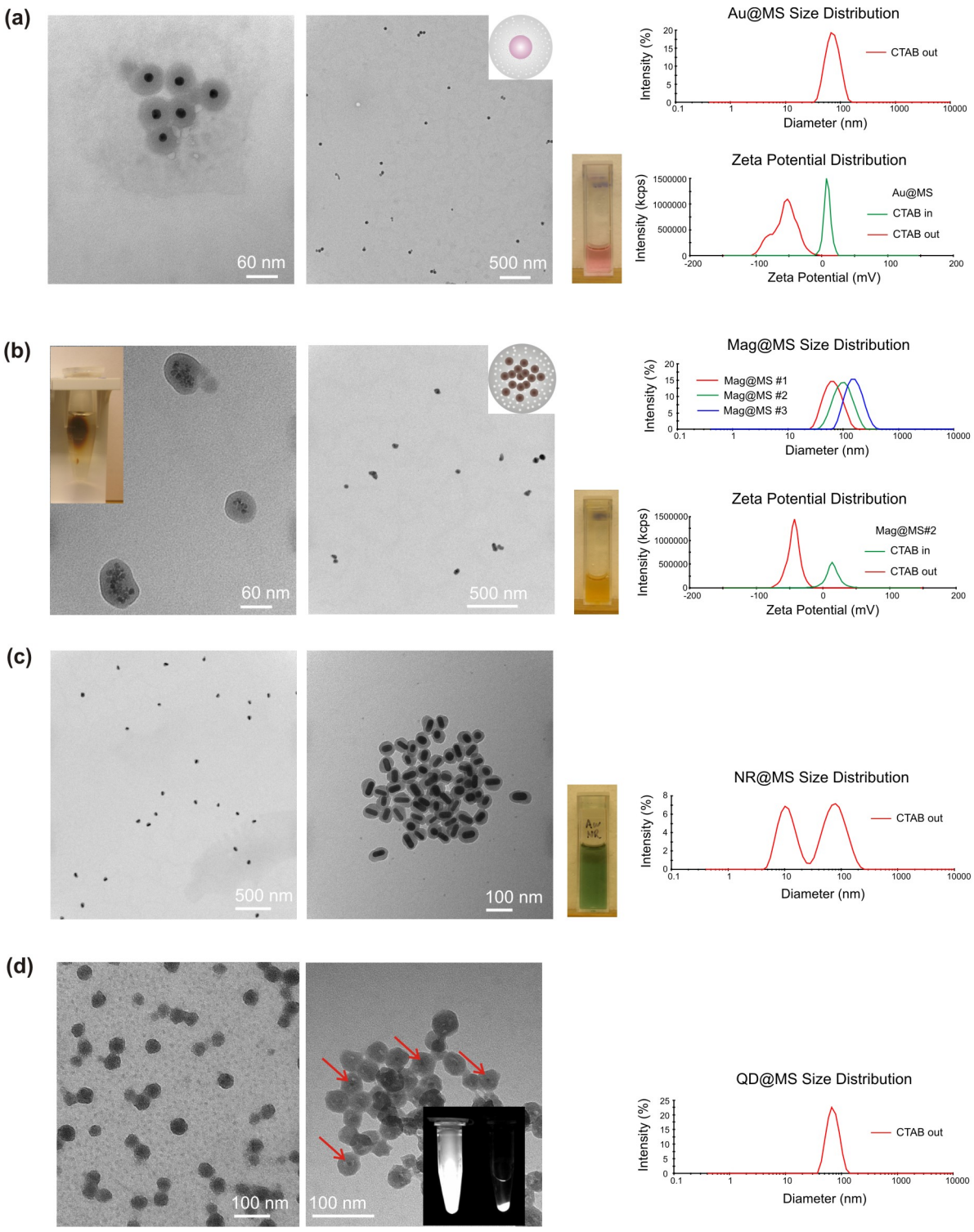
BAEC cells dye release experiments

Bovine aorta endothelial cells (BAEC) cells were plated on glass coverslips in DMEM supplemented with 10% FCS. After cell attachment, they were incubated overnight with 20 ug/mL Alexa546-loaded PEI-PEG MSN. Following NP uptake, cells were washed X3 in D-PBS supplemented with 10% glucose and imaged. Cells were exposed to TRITC-filtered light for 3 s, and immediately scanned under confocal microscopy at fast scanning rates (580 ms/frame) with simultaneous acquisition of

fluorescence and DIC images for observation of individual vesicle rupture events. The movies obtained were analyzed using Image-J (NIH).

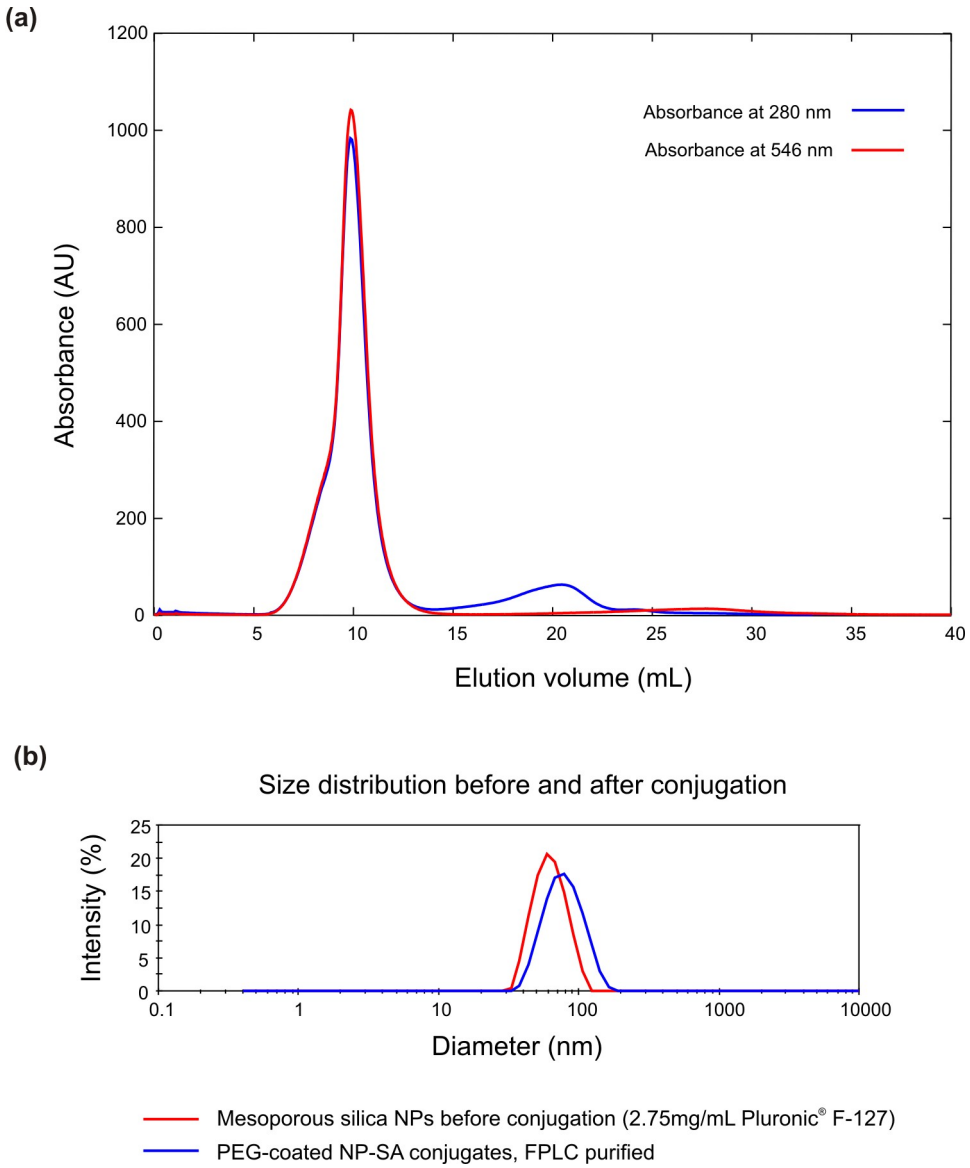
Data analysis

Confocal micrographs were analyzed using the software package Image-J. Average cell fluorescence was determined by manual contouring of cell borders. Data were then imported into Matlab (The Mathworks) for analysis and plotting purposes. The amount of dye released in the cell cytosol following light stimulation was taken as the difference in average cell fluorescence before and after light exposure.



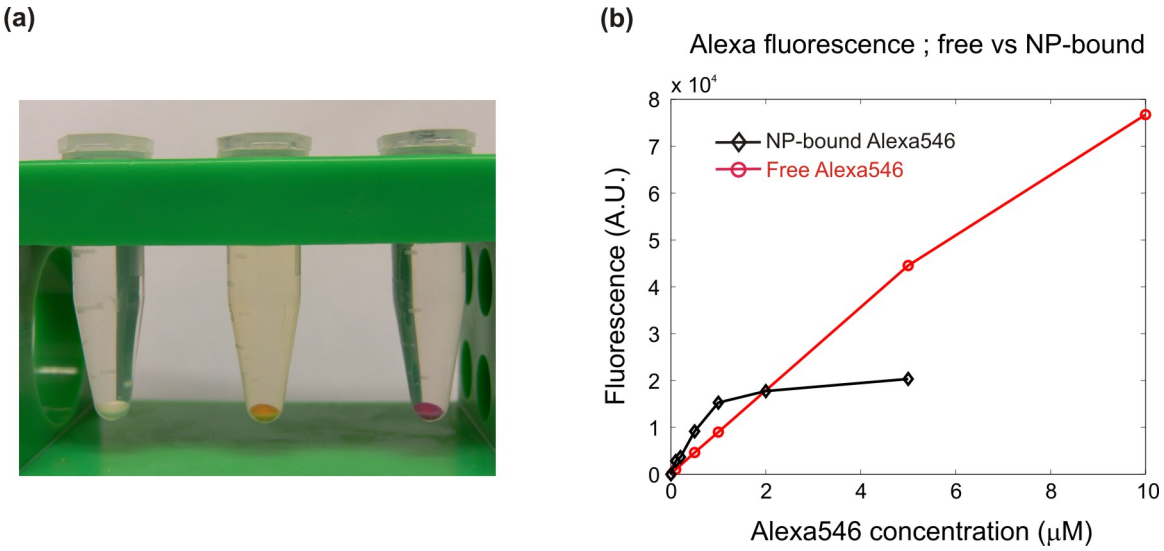
Supplementary Figure S1. Application of double-surfactant templated synthesis to various types of core-mesoporous silica shell nanostructures. (a) Gold core. Left: transmission electron micrographs of the core-shell NPs after surfactant extraction. Middle: photograph of a suspension of the NPs. Right: intensity-based DLS size distribution (top) and zeta potential distribution (bottom) before (green trace) and after (red trace) surfactant extraction. (b) Magnetic core. Left: transmission electron micrographs

of the core-shell NPs after surfactant extraction, and photograph illustrating magnetic collection of the NPs (inset). Middle: water suspension of NPs. Right: intensity-based DLS size distribution of 3 types of core-shell NPs obtained by varying synthetic conditions (top), and zeta potential distribution (bottom) before (green trace) and after (red trace) surfactant extraction. (c) Gold nanorod core. Left: transmission electron micrographs of the core-shell NPs after surfactant extraction. Middle: water suspension of NPs. Right: intensity-based DLS size distribution. (d) Quantum dot core. Left: transmission electron micrographs of surfactant-extracted NPs. Red arrows point to individual QD cores; 1 QD/NP. Inset: the core-shell NPs before and after centrifugation under UV illumination. Right: intensity-based DLS size distribution of the NPs in water.

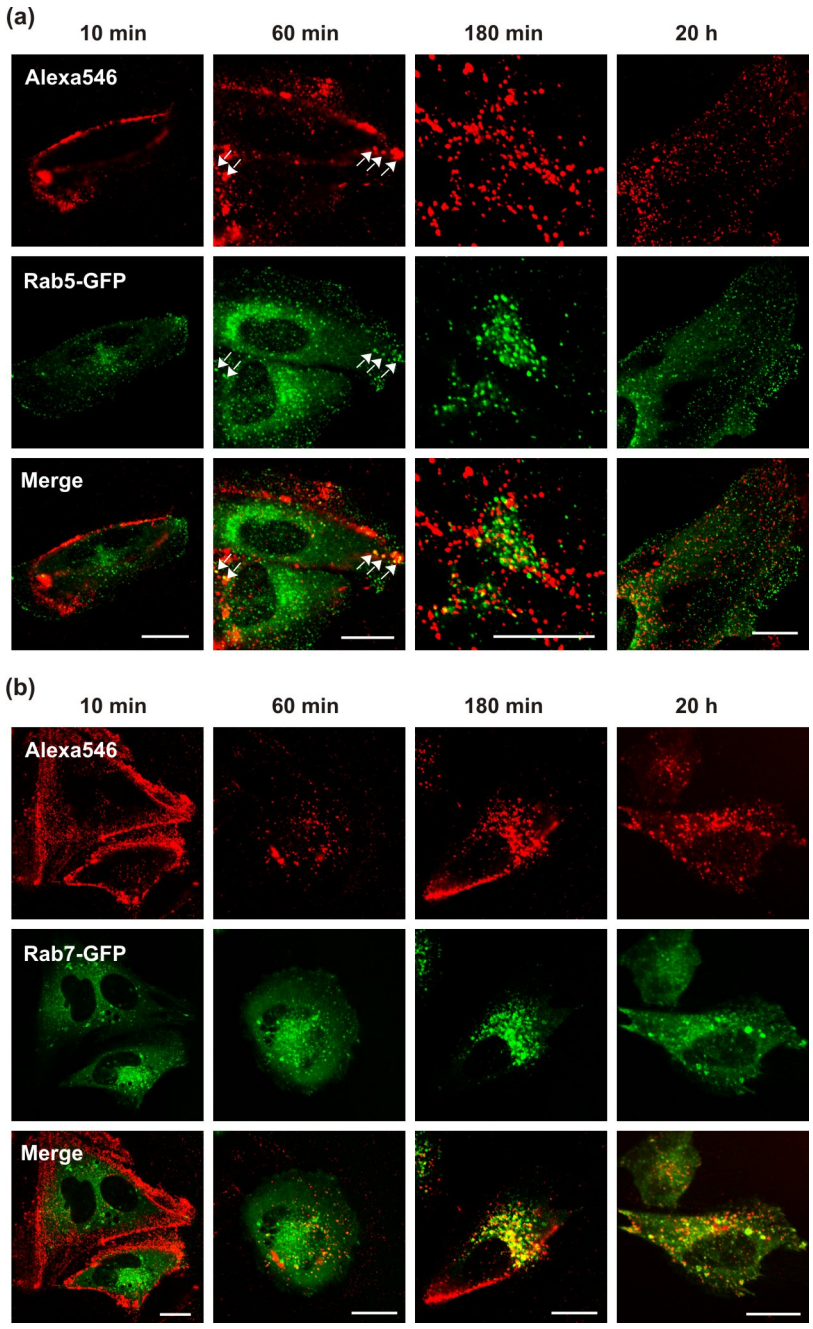


Supplementary Figure S2. Nanoparticle functionalization. (a) Size exclusion chromatogram of a 1 mL functionalized NP sample on a Sephacryl® S-400 column (30 cm length, 1 cm diameter, flow rate of 0.4 mL/min). Elution peaks are in order: NP-conjugate, unreacted streptavidin, excess PEG-maleimide, and unbound Alexa546 dye. (b) DLS size distributions of NPs before (red trace) and after

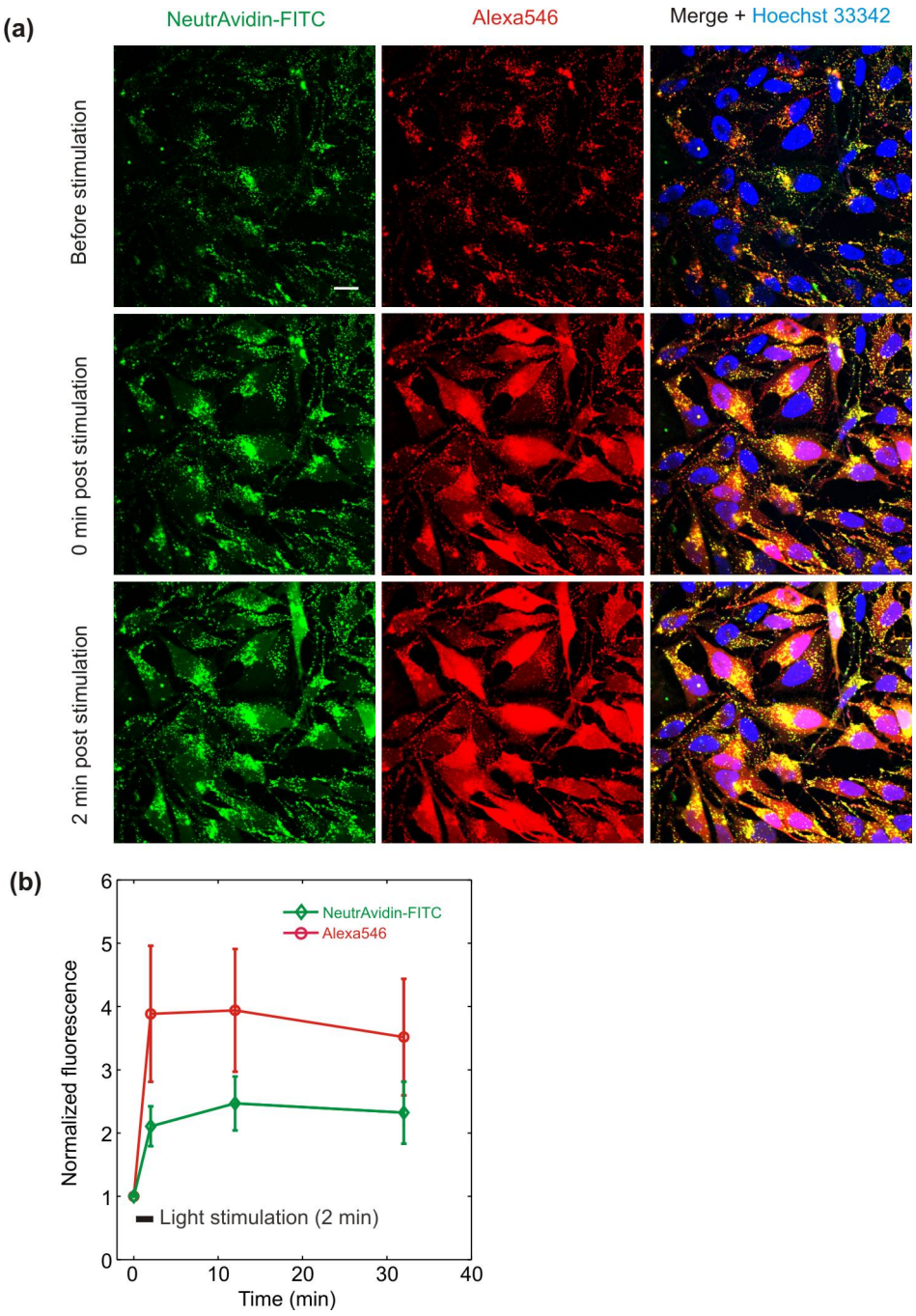
(green trace) biofunctionalization. As expected, conjugation resulted in an increase in hydrodynamic diameter. However, the limited increase in size as well as low polydispersity indicates minimal aggregation during the process.



Supplementary Figure S3. Loading and fluorescence properties of compounds adsorbed onto the silica surface. (a) Photograph of mesoporous silica NPs (100 nm diameter here) loaded with various compounds. NPs were pelleted by centrifugation at 11,000 rpm for 30 min. The compounds loaded are, from left to right: empty NPs, Tris-(2,2'-bipyridyl)-ruthenium(II) (Sigma), X-Rhod1 calcium indicator (Invitrogen). (b) Fluorescence of Alexa546 maleimide measured by plate reader (Perkin-Elmer) at various concentrations, either free (red trace) in phosphate buffered saline (PBS, 10 mM, pH 7.4), or bound to PEG-coated mesoporous silica NPs in PBS (black trace). The graph shows how association of the dye molecules with the silica matrix provides increased brightness at lower concentrations, while self-quenching occurs at higher loading rates. The amount loaded for targeting and dye delivery experiments corresponds to a concentration of 1 μM on this graph, at the upper limit of the linear range, equivalent to a 1.6% w/w ratio.

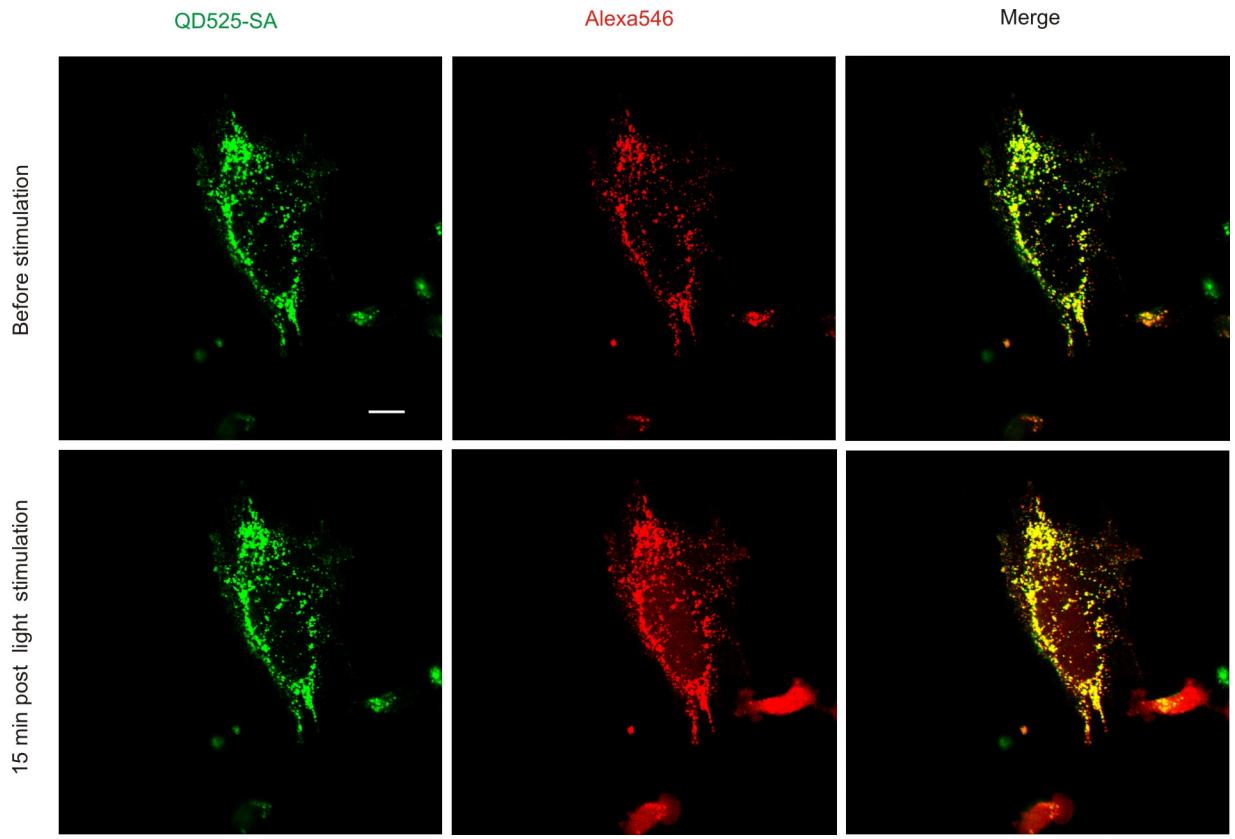


Supplementary Figure S4. Time course of PEI-PEG NPs endocytosis. Confocal micrographs of live LN-229 cells transiently transfected with the early endosome marker Rab5-GFP (a) and late endosome marker Rab7-GFP (b) following incubation with PEI-PEG functionalized NPs. Images show representative cells at different time points after incubation at 37° C. Early endosomes containing NPs can be observed at 60 min (arrows), while NPs can be observed in Rab-7 tagged vesicles at later time points (180 min). Scale bars are 20 microns.

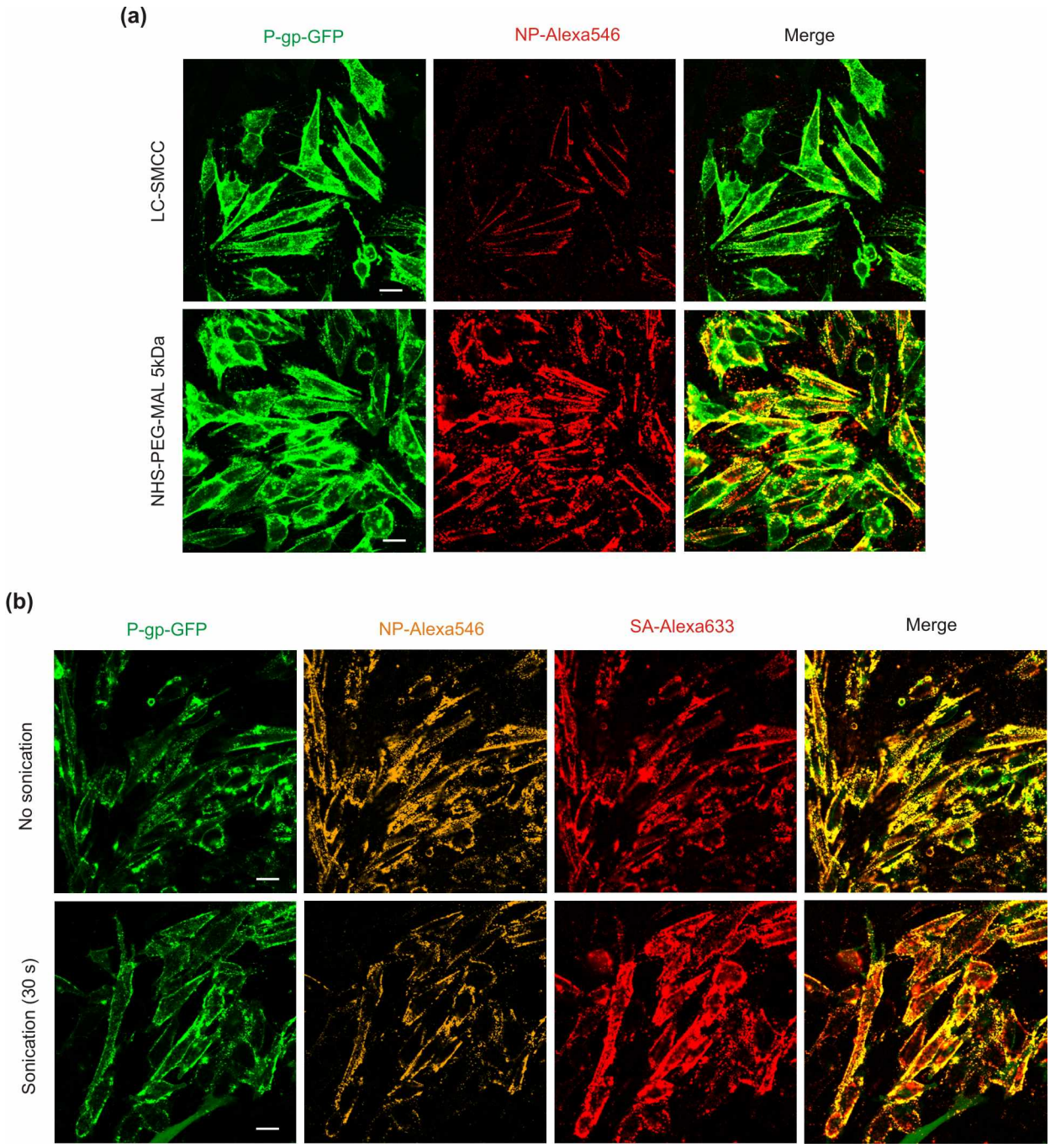


Supplementary Figure S5. NeutrAvidin release experiments. (a) Confocal micrographs of LN-229 cells after NP and NeutrAvidin-FITC co-endocytosis (60X water-immersion objective, scale bar 20 microns). NPs were loaded with Alexa546. Images were acquired before (top row), immediately after (middle row) and 2 min following 120 s light stimulation of the NPs. Light stimulation caused rapid and massive extrusion of the loaded Alexa546 dye. The endocytosed NA-FITC conjugates, largely colocalized with NPs in the endosomal compartment before light exposure, were released in the cell cytosol as well, with slower kinetics than the dye, as expected. (b) Time evolution of Alexa546 and NeutrAvidin-FITC average cell fluorescence (normalized to initial fluorescence) following light stimulation, reflecting a significant increase for both. While the maximum fluorescence was reached

before the end of light stimulation (120 s) for the Alexa dye, the maximum fluorescence for the NA-FITC conjugate is only reached after >10 min following the end of light exposure.



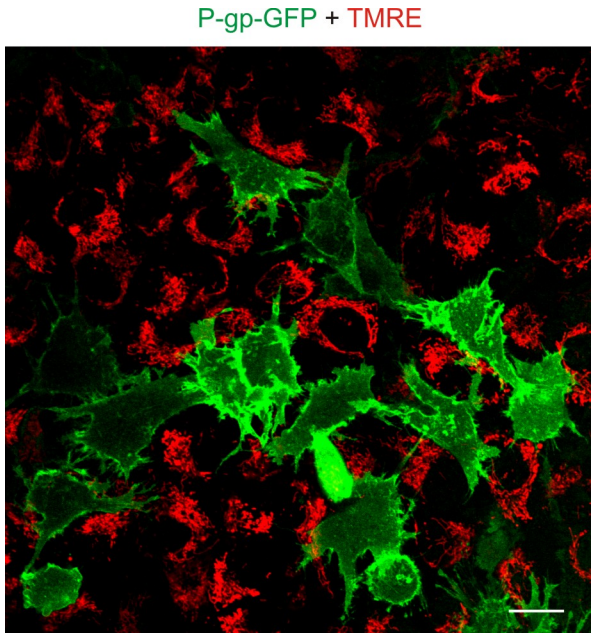
Supplementary Figure S6. Quantum dot release. Confocal micrographs of LN-229 cells after NP and QD525-streptavidin co-endocytosis. Images were acquired before (top row), and 15 min after 120 s light stimulation (60X water-immersion objective, scale bar 20 microns). In contrast to NP-released Alexa546, the QDs do not migrate into the cytosol of the cells following stimulation of the co-endocytosed NPs over a period of >30 min. This may be due to irreversible clustering of the QDs following endocytosis, or multiple streptavidin-biotin bonds for each QD, preventing separation from the endosomal compartment.



Supplementary Figure S7. Effect of crosslinker length and nanoparticle sonication on targeting efficiency. (a) Confocal micrographs of LN-229 cells stably expressing P-gp-GFP after incubation with NPs functionalized with different crosslinkers. The 2 NP types tested are identical in size (2.75 mg/mL Pluronic® F-127 in the reaction mixture), functionalized with the same amount of streptavidin (60 µg SA per 2 mg NPs), but with two different crosslinkers: LC-SMCC (Pierce Biotechnology, 15:1 molar ratio to SA, top row) and NHS-PEG-MAL 5kDa (Rapp Polymere, 5:1 molar ratio to SA, bottom row). Images were acquired using identical settings. We speculate that the superior targeting efficiency was obtained with the longer crosslinker due to increased mobility and reduced steric hindrance for the immobilized streptavidin. (b) Confocal micrographs of LN-229 cells stably

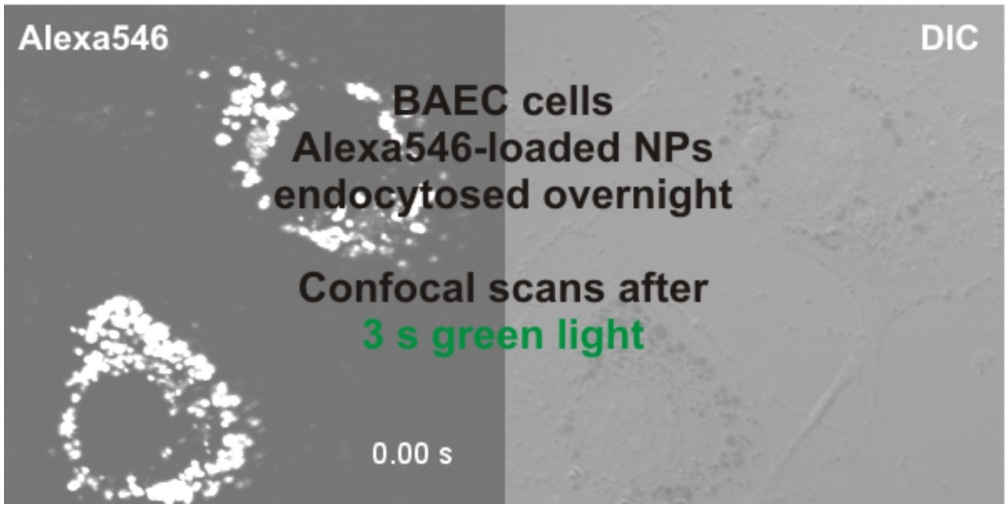
expressing P-gp-GFP after incubation with identical NPs, either sonicated for 30 s before incubation (bottom row), or without sonication (top row). All samples were imaged using a 60X water-immersion objective. Scale bars are 20 microns.

The NPs used in this experiment were conjugated to a fluorescent streptavidin conjugate (SA-Alexa633, Invitrogen) with a PEG-5kDa spacer before loading with Alexa546. Images were acquired using identical parameters. For the non-sonicated NPs, the ratio of 633 to 546 fluorescence is ~ 1. As evidenced by the strong red signal in the overlay image, the SA-633 fluorescence is significantly higher than the Alexa546 signal for the sonicated NPs. This suggests that the detachment of streptavidin from the nanoparticles was due to the sonication process, possibly restricted to non-covalently bound streptavidin adsorbed on the silica surface by way of its associated PEG linker. It is also possible that covalently bound streptavidin may be displaced from the NP surface. Due to their significant size difference, the free streptavidin saturates binding sites on the cell surface before NPs can interact. This confirms the utility of a chromatography-based purification technique to remove unbound biomolecules following functionalization, as opposed to cycles of centrifugation/resuspension that usually require sonication.

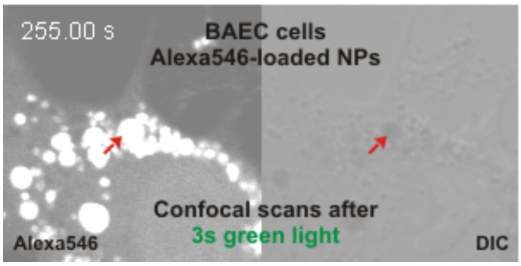


Supplementary Figure S8. Characterization of the P-glycoprotein-GFP fusion by substrate loading. Confocal micrograph of transiently transfected cells (LN-229) expressing the construct after incubation with tetramethyl-rhodamine-esther (TMRE, 50 nM in D-PBS-glucose) for 10 min at room temperature (scale bar 20 microns). P-glycoprotein-GFP-expressing cells show complete extrusion of TMRE, while nontransfected cells display high dye loading with a typical mitochondrial distribution pattern. Peri-membrane localization of the construct is observed, and membrane localization was confirmed by live cell antibody staining to an extracellular epitope (BD, same primary antibody used for NP targeting experiments, data not shown).

Supplementary Movie Legends



Supplementary Movie S1. Individual vesicle disruption events can be observed by laser confocal scanning microscopy. After overnight endocytosis of PEG-PEI NPs loaded with Alexa546 (20ug/mL), BAEC cells were exposed to TRITC filtered light for 3 s (60X water-immersion objective), and monitored by continuous confocal scanning at a rate of 580 ms/frame.



Supplementary Movie S2. Vesicle disruption in the vicinity of a dendritic process. The experimental conditions are identical to *Supplementary Movie S1*. A massive vesicle disruption can be observed (indicated by the red arrows), causing immediate release of the dye cargo into the dendritic process, followed by a rapid diffusion across the entire cell cytosol. The DIC image shows a significant decrease in light absorption across the vesicle upon release.

Supplementary References

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