

Supporting Information for:

Size-Dependent Electroporation of Dye-Loaded Polymer Nanoparticles for Efficient and Safe Intracellular Delivery

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Optimization of Electroporation Conditions

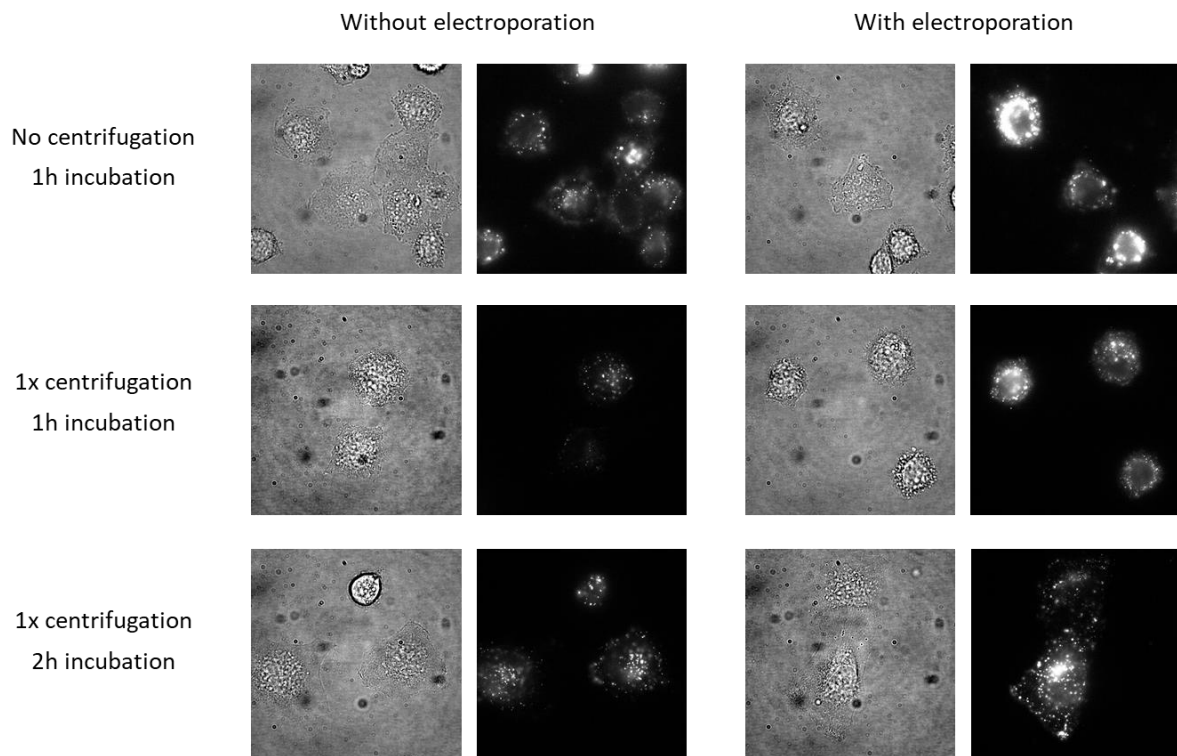


Figure S1. Influence of centrifugation and incubation time. DIC (left) and fluorescence (right) micrographs of cells treated with 13 nm NPs. The left and right columns correspond to conditions without and with electroporation, respectively. The effect of a centrifugation step directly after electroporation or bringing the cells in contact with the NP solution and of the incubation time following this treatment is evaluated. $\lambda_{\text{ex}} = 550 \text{ nm}$, $\lambda_{\text{em}} = 555 - 635 \text{ nm}$. Scale bars: 20 μm .

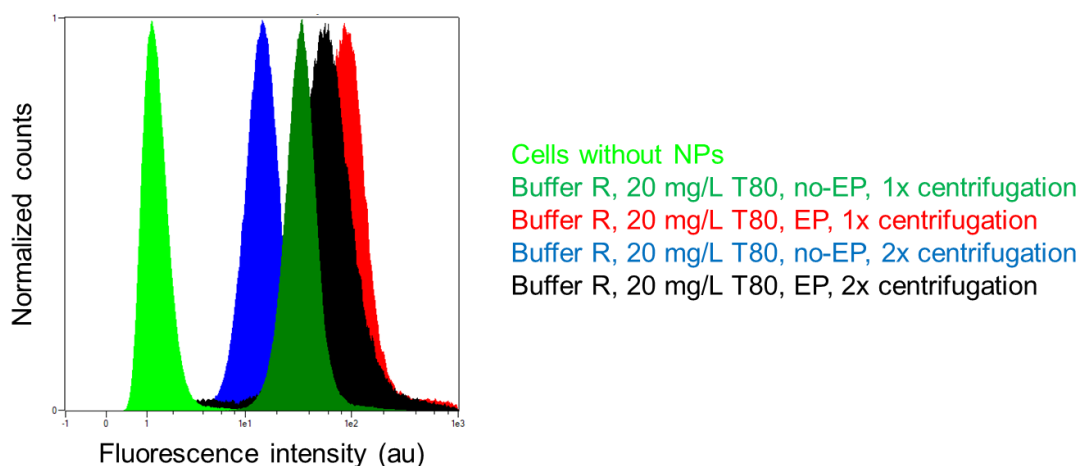


Figure S2. Influence of a second centrifugation step as determined using flow cytometry. Cells were treated with 13 nm NPs with and without electroporation, followed directly by 1 or 2 centrifugation steps. The cells were then immediately analyzed using flow cytometry with a 561 nm for excitation and a 651/671 nm band pass filter for the emission.

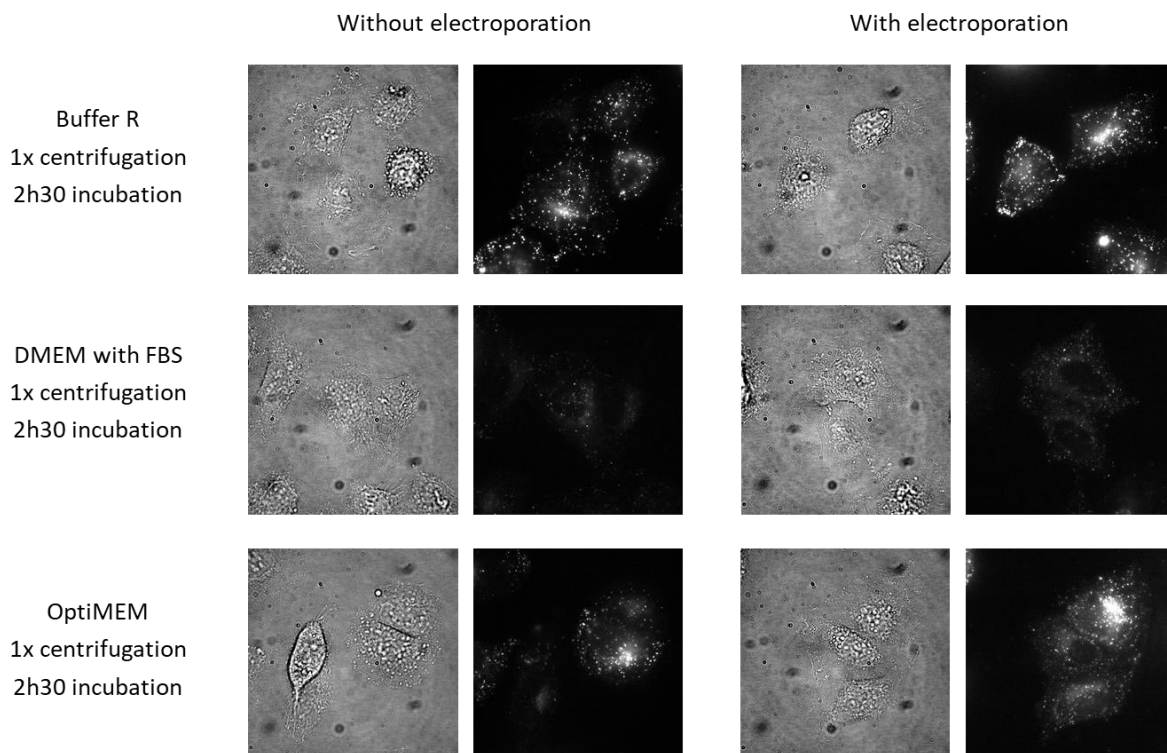


Figure S3. Effect of the electroporation medium. DIC (left) and fluorescence (right) micrographs of cells treated with 13 nm NPs. The left and right columns correspond to conditions without and with electroporation, respectively. The effect of different media was evaluated, in which the cells were brought in contact with the NP solution, and, if applicable, electroporated. DMEM with FBS gave lower signals for both, electroporated and non-electroporated cells. OptiMEM gave larger cell to cell variations. $\lambda_{ex} = 550 \text{ nm}$, $\lambda_{em} = 555 - 635 \text{ nm}$. Scale bars: 20 μm .

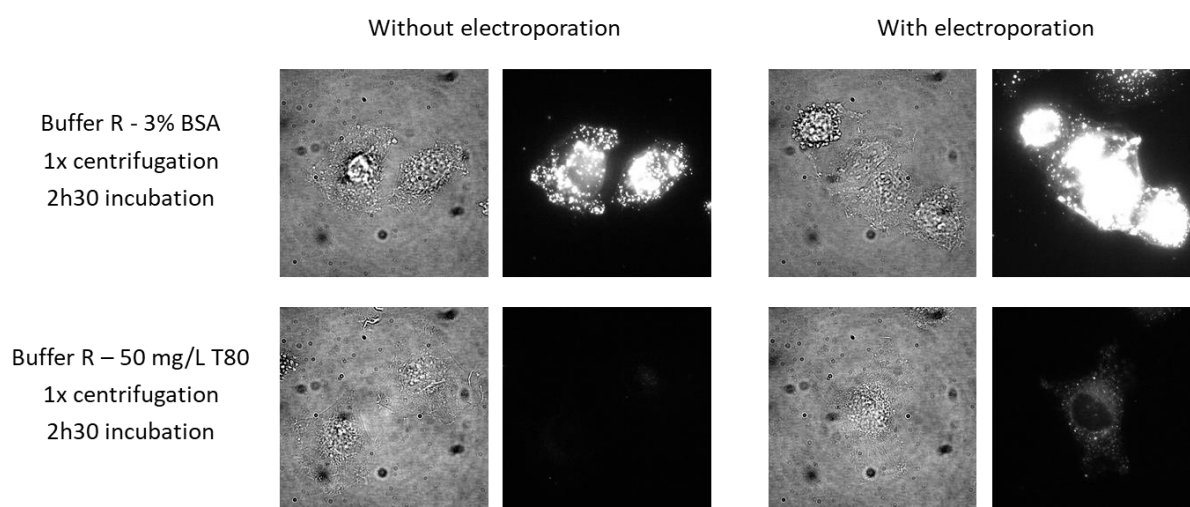


Figure S4. Control of interactions. DIC (left) and fluorescence (right) micrographs of cells treated with 13 nm NPs. The left and right columns correspond to conditions without and with electroporation, respectively. The effect of two blocking agents, bovine serum albumin (BSA) and Tween 80 (T80), added to the medium in which the cells were brought in contact with the NP solution, and, if applicable, electroporated, was evaluated. Tween 80 led to a strong reduction of NP endocytosis in the absence of electroporation. $\lambda_{ex} = 550 \text{ nm}$, $\lambda_{em} = 555 - 635 \text{ nm}$. Scale bars: 20 μm .

Single Particle Tracking

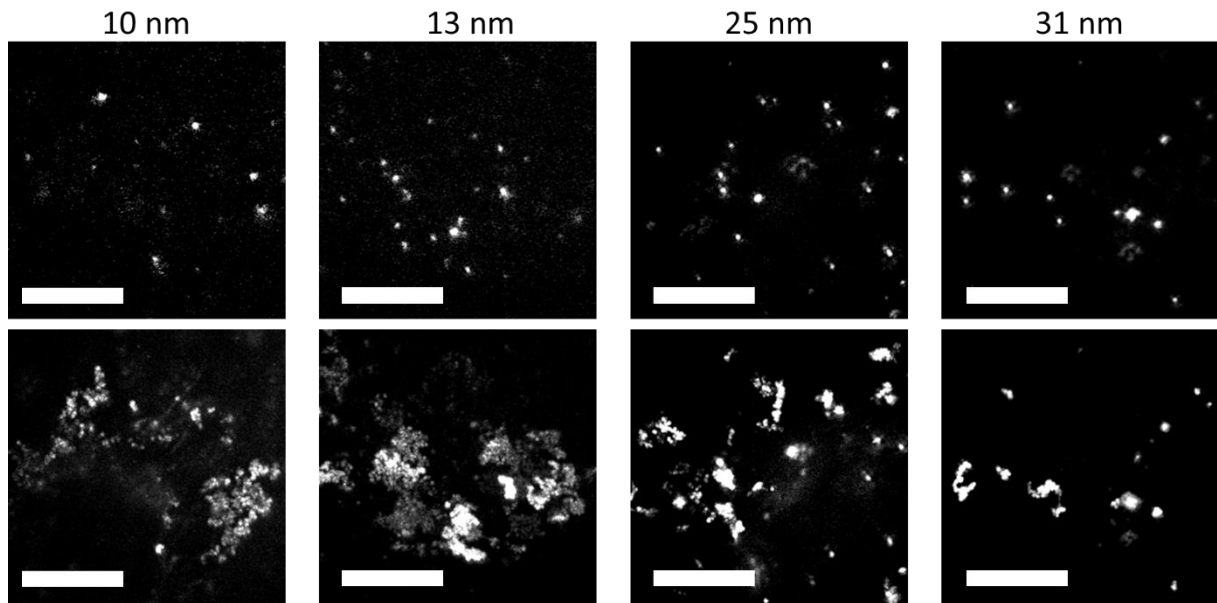


Figure S5. Examples of fluorescence micrographs of cells electroporated with NPs of different sizes used for single-particle tracking. Top: Instantaneous fluorescence. Bottom: Maximum projections over 30 s. $\lambda_{\text{ex}} = 532 \text{ nm}$, $\lambda_{\text{em}} = 555 - 635 \text{ nm}$. Scale bars: $10 \mu\text{m}$.

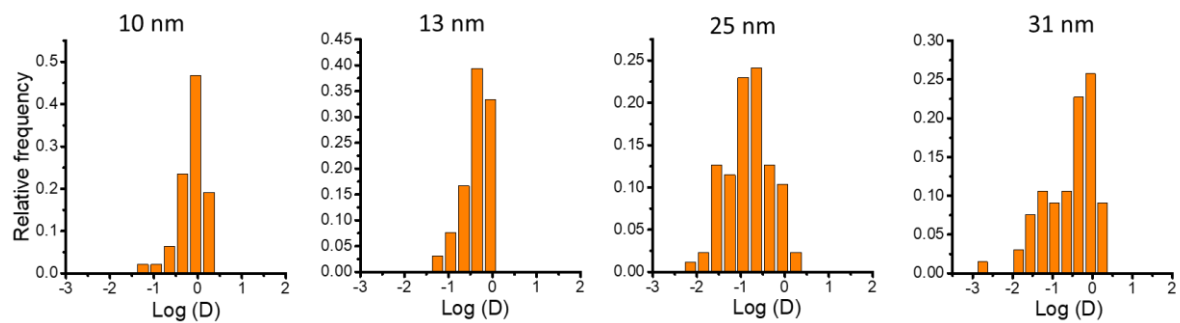


Figure S6. Distribution of diffusion coefficients determined for NPs of different sizes in HeLa cells. At least 30 trajectories were analyzed per sample.

Influence of Electroporation Conditions

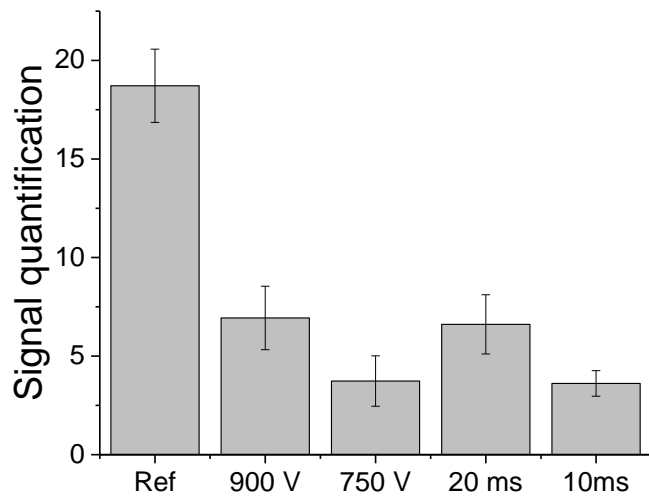


Figure S7. Average fluorescence intensity per cell obtained for different electroporation conditions with 13 nm NPs. Reference conditions are 2 pulses of 35 ms with 1050 V. Either pulse amplitude or length were varied as noted. At least 20 cells per condition were evaluated.

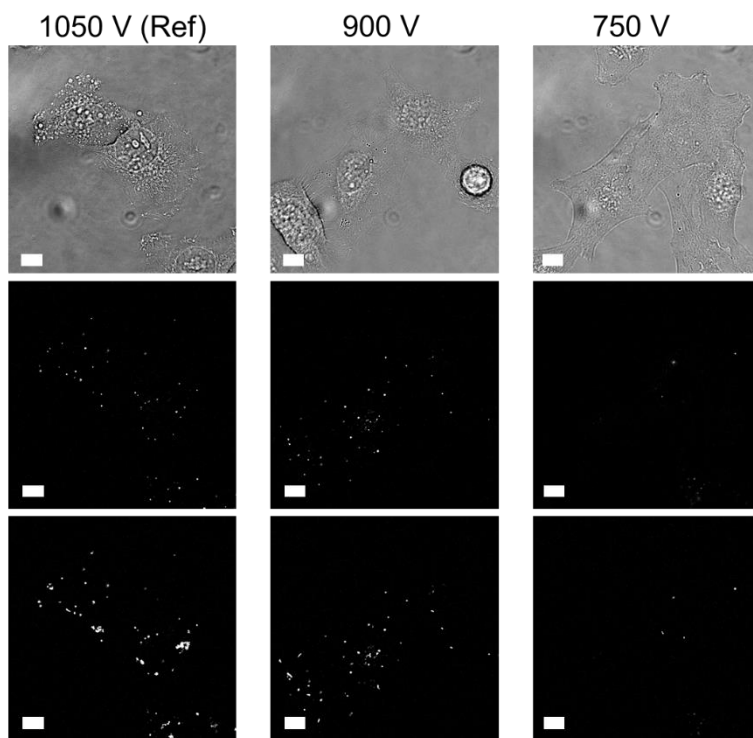


Figure S8. Micrographs of cells electroporated using different pulse intensities with 38 nm NPs. Top: DIC. Middle: Instantaneous fluorescence image. Bottom: Maximum projections over 36 s. $\lambda_{\text{ex}} = 550$ nm, $\lambda_{\text{em}} = 555 - 635$ nm. Scale bars: 10 μm .