

Size-dependent and Real-Time Effect of SiO₂ Nanoparticles on a Single Living HeLa Cell Membrane Permeability (Supporting Information)

Reagents and Materials. All the chemicals were purchased from Sigma-Aldrich and used as received. The reagents were all analytical purity. All solutions were made with Milli-Q water (Millipore).

Characterization Methods Scanning electron microscopy (SEM) images were taken on a FEI-quanta 200 scanning electron microscope with acceleration voltage of 20 kV. The transmission electron microscopy (TEM) images were obtained with a Tecnai G2 F20 electron microscope at an acceleration voltage of 200 kV. The Fourier Transform Infrared (FT-IR) spectrum of SiO₂ NPs was characterized with a Varian Spectrum GX spectrometer.

Preparation of 200 nm SiO₂ NPs. The 200 nm SiO₂ NPs were prepared according to Stöber synthesis. In a typical synthesis, 3 mL 25% NH₃·H₂O were added into 25 mL 95% ethanol with magnetic stirring at room temperature. Then 2 mL 98% tetraethyl orthosilicate (TEOS) were dripped into the mixed solution. The solution turned white after 20 min. After aged for overnight, the mixed solution was centrifuged at 5000 rpm for 5 min. Then the white precipitate was washed three times with ethanol and deionized water, respectively. The pure white precipitate was dried at 60 °C and the 200 nm SiO₂ NPs were obtained.

Preparation of 100 nm SiO₂ NPs. In the experiment, 1 mL 25% NH₃·H₂O were added into the mixed solution of 10 mL 95% ethanol and 5 mL H₂O with magnetic stirring at room temperature. 0.5 mL TEOS were dropped into the mixed solution and continued stirring for 24 h. The obtained solution was centrifuged at 8000 rpm for 5 min. Then the white precipitate was washed three times with ethanol and deionized water, respectively. The pure white precipitate was dried at 60 °C and the 100 nm SiO₂ NPs were obtained.

Preparation of 50 nm SiO₂ NPs. In the experiment, 0.5 mL 25% NH₃·H₂O were added into the mixed solution of 12 mL 95% ethanol and 8 mL H₂O with magnetic stirring at room temperature. Then 0.5 mL TEOS were dropped into the mixed solution and continued stirring for 24 h. The obtained solution was centrifuged at 12000 rpm for 5 min. Then the white precipitate was washed three times with ethanol and deionized water, respectively. The pure white precipitate was dried at 60 °C and the 50 nm SiO₂ NPs were obtained.

Preparation of rhodamine B-in-SiO₂ NPs The rhodamine B-in-SiO₂ NPs were prepared by a two-step method. First, TEOS was slowly added to the ethanol solution. The volume ratio of the reagents for preparing different sizes of rhodamine B-in-SiO₂ NPs are the same with that of preparing different size of SiO₂ NPs. Second, the 1 mmol rhodamine B (based on the ethanol solution) was added into the previous solution and a precipitate started to form as the reaction proceeded. The obtained precipitates were washed for several times with deionized water until the surface of the precipitate has no rhodamine B. Then, the rhodamine B-in-SiO₂ NPs were synthesized.

Preparation of FCDs-SiO₂ NPs The FCDs-SiO₂ NPs were prepared by modifying the SiO₂ NPs with FCDs. First, the FCDs were added into water. Next, SiO₂ nanoparticles (0.05 – 0.1 g) were added to the FCDs solution (5 mL) with stirring for 10 min and dried in a vacuum oven at 80 °C for 12 h to give the FCDs-SiO₂ NPs.

Cell Culture. The HeLa cells were grown as a monolayer and incubated in a water-jacketed incubator (Thermo, USA) with 5% CO₂ and the temperature maintained at 37 °C. The HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Beijing) supplemented with 10% fetal bovine serum (FBS) and 1% mixed solution of penicillin and streptomycin (Gibco, Hangzhou). The coverage of HeLa cells was observed with an Olympus (CKX41, Japan) inverted microscope. In order to obtain more adherent single cells, the HeLa cells were incubated for 12 h. Then the cellular monolayers were washed three times with PBS (phosphate buffer solution, pH=7.4). After that, the cells were incubated with PBS at room temperature. During the experiment, the PBS was replaced by 4 mM ferrocyanide PBS or different kinds of SiO₂ NPs solution.

Electrochemistry and electrode fabrication. An inverted digital microscopy was used to observe the coverage and living single HeLa cell. SECM data were obtained with the CHI 920C (Shanghai, China) workstation which was fixed an inverted microscopy and used an inverted microscopy to observe the cell coverage and the states of HeLa cells. In addition, the three-electrode system of SECM was used in our laboratory which the ultramicroelectrode of 25 μ m diameter platinum wire disk was used as working electrode, a 1mm platinum wire was used as counter electrode and the Ag/AgCl was used as reference electrode. All the potential here were reference to Ag/AgCl.

Fluorescence Based Cell Viability. In order to discuss the cytotoxicity of SiO₂, calcein-AM and PI were used in this assay. This is a useful method to measure both live and dead cells at the same time. Calcein-AM is a cell-permeant and non-fluorescent compound. Calcein-AM could only across an intact cell membrane and react with the intracellular esterase then the AM group dropped and producing a highly fluorescent membrane impermeant green fluorophore (excitation: 490 nm, emission: 515 nm). So the calcein-AM is widely used for determining cell viability. On the contrary, PI can't pass through living Hela cell membrane but can across the damaged membrane and react with intracellular nucleic acid to emit red fluorescence inside dead cells (excitation: 535 nm, emission: 617nm). So its red fluorescent was used as a nuclear or chromosome counterstain; stain for dead cells. These emitted fluorescence cells were easily visible though fluorescence microscopy (Nikon). In this experiment, 10 μ L calcein-AM and 15 μ L PI were dissolved into 5 mL PBS, the concentration of calcein-AM and PI are 2 μ mol/L and 4 μ mol/L, respectively. The different size of SiO₂ NPs and HeLa cells were co-cultured for 12 h. Then the mixed solution of dyes was added cultured for 15 min. Then the toxicity test cell viability was measured in real time.

Approach curves over a single cell. All of approach curves were obtained in the 4 mMferrocyanide PBS with a potential of 0.5 V. The current rate was set as 80%. Then the solution was replaced by SiO₂ NPs solution with 4 mM ferrocyanide. The approach curves can also be measured for the cell topographic information.

X-scan over a single cell. The HeLa cells were cultured on a 35 mm Petri dish until the cell coverage was about 50%. The approach curves were measured at three different points outside the cells in 4 mM ferrocyanide with PBS. Then the stage of SECM was adjusted to make sure the stage is level. With the help of an inverted microscopy, a single living cell can be found. The SECM tip was positioned above the cell about 20 μm . The x-scan lines can be measured in a constant height with an increase distance of 0.2 μm . When a single cell was identified, different size of SiO_2 NPs solution with 4 mM ferrocyanide buffer solution was added into the solution to replace the previous solution. And a series of x-scans lines were obtained over this single cell to investigate the effect of different size of SiO_2 NPs on cell membrane permeability.

i-t curves over a single cell. By approach curves the tip was local above the cell. Then the tip position was adjusted to make the tip hold at 10 μm above a single cell with a constant potential of 5.0 V. And the *i-t* curve near the cell membrane was obtained.

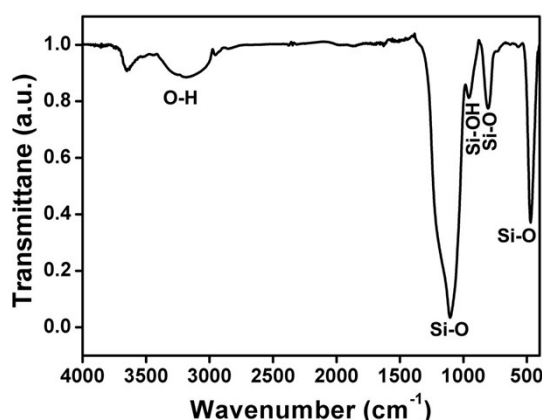


Figure S1. The FT-IR spectrum of 50 nm SiO_2 NPs.

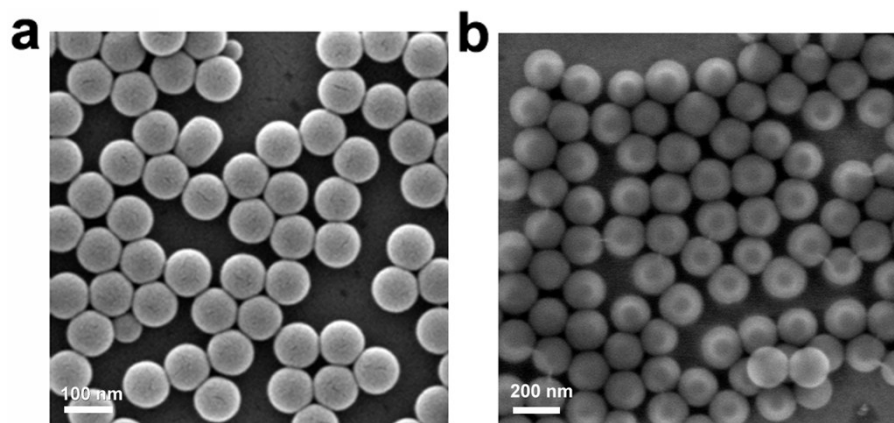


Figure S2. (a) and (b) SEM images of 100 nm and 200 nm SiO₂NPs, respectively.

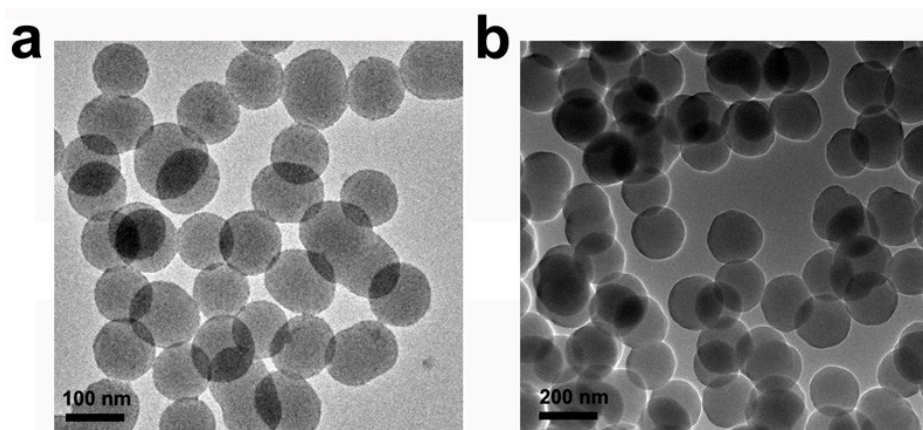


Figure S3. (a) and (b) TEM images of 100 nm and 200 nm SiO₂NPs, respectively.

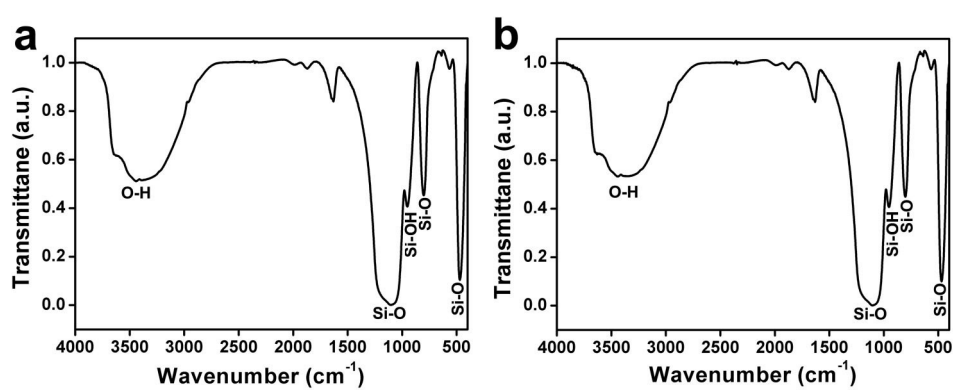


Figure S4. (a) and (b) FT-IR spectra of 100 nm and 200 nm SiO₂NPs, respectively.

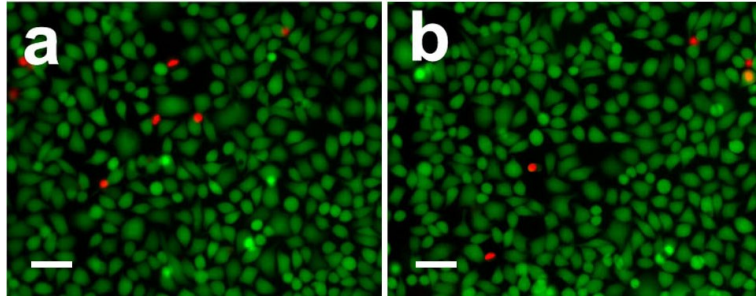


Figure S5. (a and b)Fluorescent images of 100 nm and 200 nm SiO₂ NPs, respectively. The green color represents living cells and the red color represents the dead cells. Scale bar=100 μm.

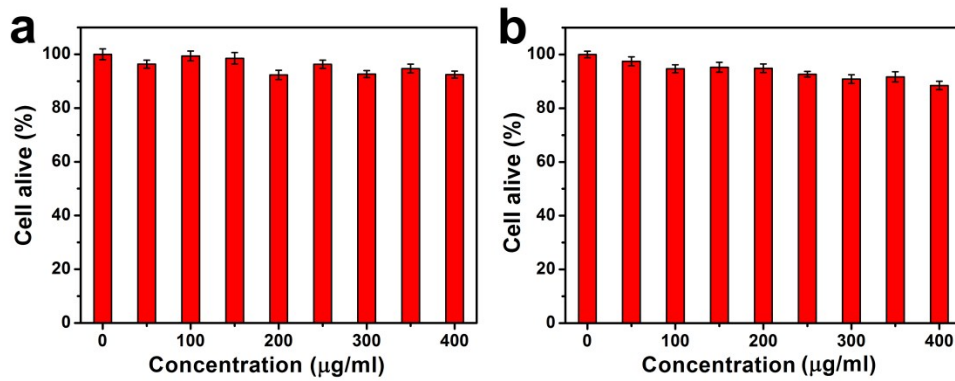


Figure S6. (a and b)Fluorescence based viability to HeLa cells for different concentrations of 100 and 200 nm SiO₂ NPs, respectively.

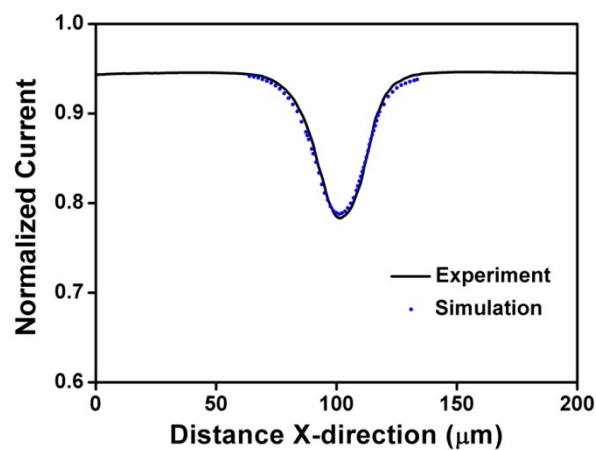


Figure S7. The experiment x-scan line above a single living cell contrast to the simulated x-scan line.

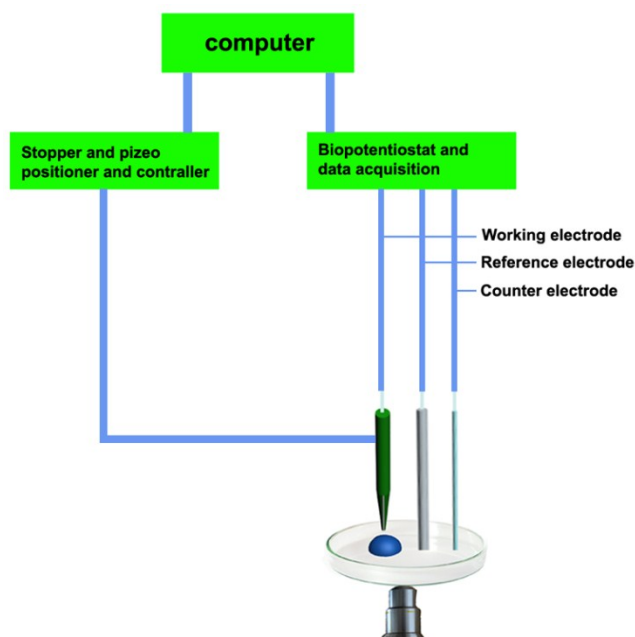


Figure S8. The schematic diagram of scanning electrochemical microscopy for measuring a HeLa cell.

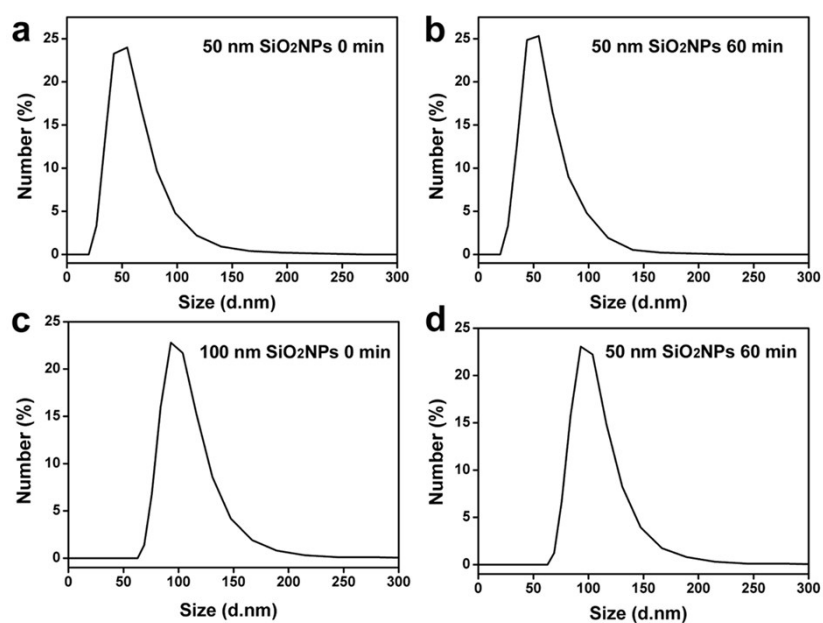


Figure S9. The DLS data of different sized SiO₂ NPs in PBS: (a) to (d) for 50 nm SiO₂ NPs (0h), 50 nm SiO₂ NPs (1h), 100 nm SiO₂ NPs(0h), and 100 nm SiO₂ NPs (1h), respectively.

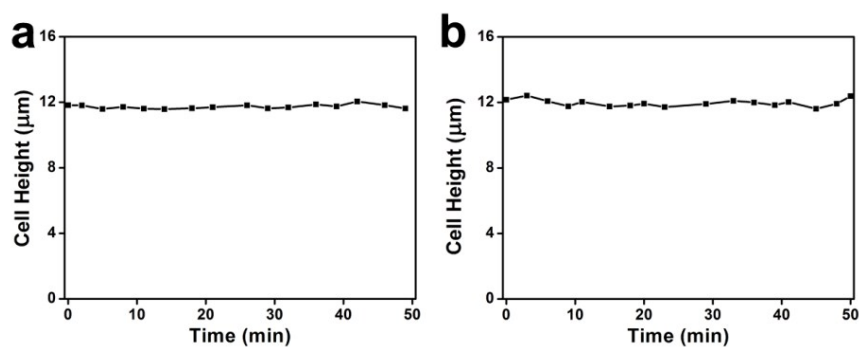


Figure S10. (a and b) The cell height in 100 and 200 nm SiO_2 NPs solution, respectively.

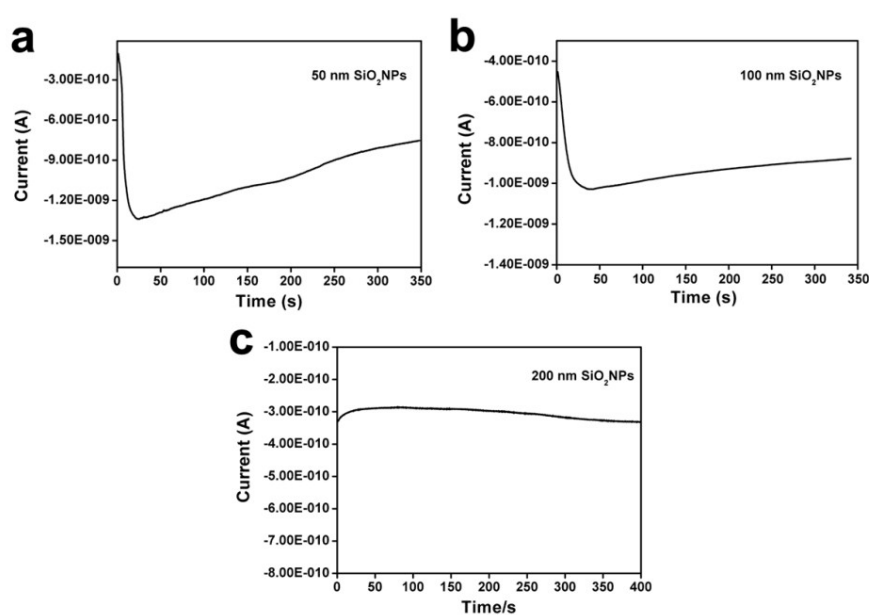


Figure S11. The tip was positioned above the cell membrane approximately $20\text{ }\mu\text{m}$ to obtain the real-time i-t curves. a, b and c are the i-t curves of HeLa cells in 0.02 mg/mL 50, 100 and 200 nm SiO_2 NPs, respectively.

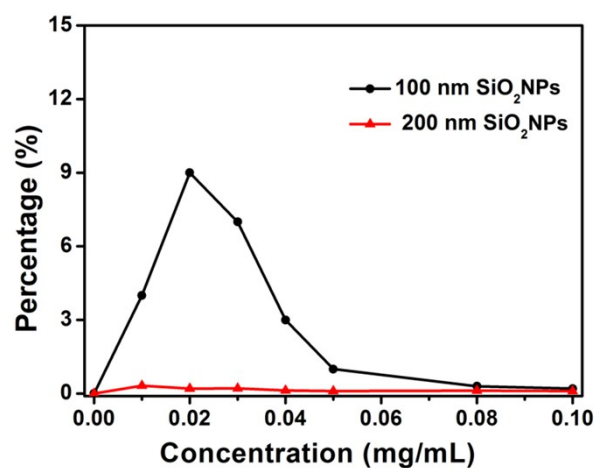


Figure S12. The plot of normalized current versus time for 100(black line) and 200 nm (red line) SiO₂ NPs, respectively.

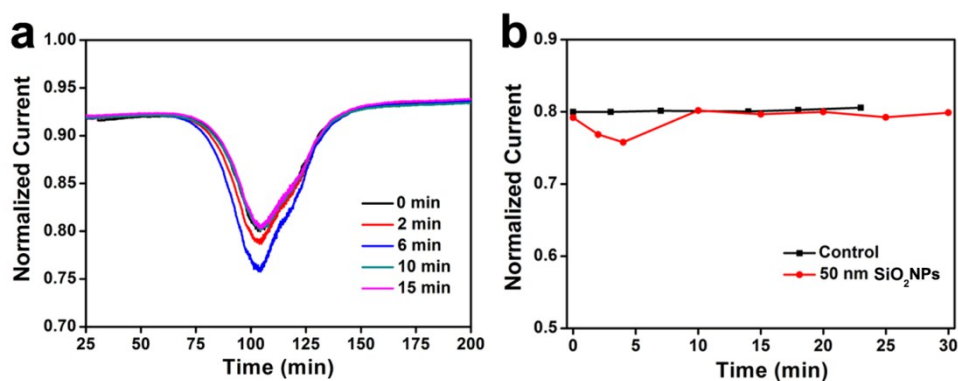


Figure S13. The x-scan lines (a) and the minimum normalized current against time (b) of a single cell in present of 0.01 mg/mL 50 nm SiO₂ NPs.

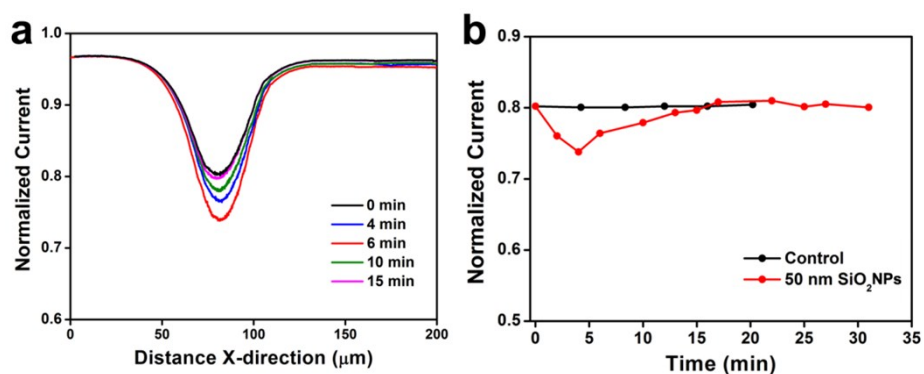


Figure S14. The x-scan lines (a) and the minimum normalized current against time (b) of a single cell in present of 0.03 mg/mL 50 nm SiO₂ NPs.

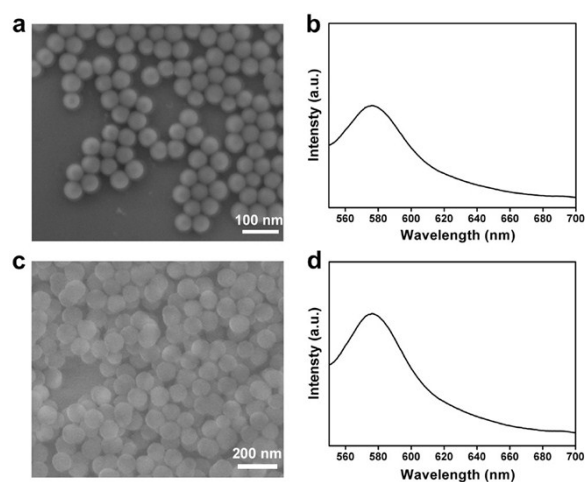


Figure S15. (a and c) SEM images of 100 and 200 nm rhodamine B-in-SiO₂ NPs, respectively. (b and d) the PL spectrum of the 100 and 200 nm rhodamine B-in-SiO₂ NPs, respectively.

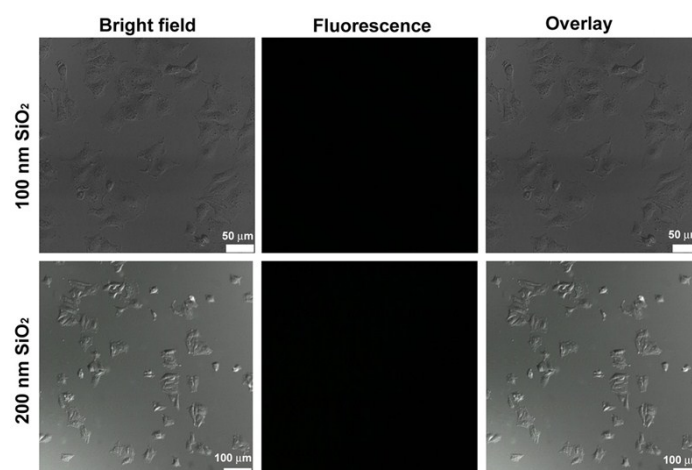


Figure S16. The confocal images of 100 and 200 nm rhodamine B-in-SiO₂ NPs stained HeLa cells, respectively.

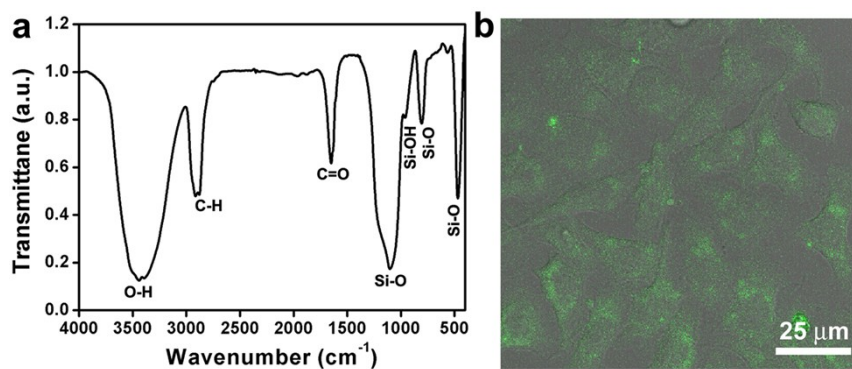
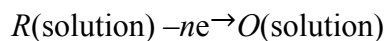


Figure S17. (a) The IR spectrum of the 50 nm FCDs modified SiO₂ NPs. (b) The

confocal images of 50 nm FCDs modified SiO₂ NPs stained HeLa cells.

Simulation

The ferrocyanide was assumed underwent one electron transfer in the experiment. And the tip was held at diffusion controlled potential to avoid any kinetics complications.



R represents ferrocyanide and O represents ferricyanide. For ferrocyanide, $n=1$.

Because the redox species R and O moved toward and away from the electrode surface only by concentration gradient Fick's second law of diffusion which was used in the simulation. The concentration of species R is given as $c_R(r, z, t)$ and the diffusion equation in cylindrical coordinates is described as

$$\frac{\partial c_R}{\partial t} = D \left(\frac{\partial^2 c_R}{\partial r^2} + \frac{1}{r} \frac{\partial c_R}{\partial r} + \frac{\partial^2 c_R}{\partial z^2} \right)$$

Where r and z are the coordinates, t represents time, c and D represents the concentration and the diffusion coefficient of R . Then the tip-current response was simulated using initial conditions and the appropriate boundary conditions. The simulation domain and boundary conditions are displayed in Figure S18.

The boundary conditions at $t > 0$

At the tip: $0 < r < a, z = h_1$

$$c_R(r, h_1) = 0$$

$$c_O(r, h_1) = 4$$

At the substrate: $0 < r < r_m, z = h_2$

$$\frac{\partial c}{\partial z} = 0$$

At the cell membrane: $arc1, d < z < h_2$

Flux of R across the $acr1 = P(R - R1)$

P represents the permeability of R across cell membrane. R and $R1$ represent the ferrocyanide outside and inside the cell, respectively.

At the beginning, the concentration of ferrocyanide inside and outside the cell was 0 mM and 4 mM, respectively. Owing to the permeability, only the ferrocyanide molecular can across the cell membrane with proper potential. And the current can be measured with a tip-substrate distance of 20 μm .

$$I_{tip} = \int_{r=0}^{r=a} 2\pi n F D_R r \frac{\partial c_R(r, h_2)}{\partial z} dr$$

where $n=1$, $F=96485$ C/mol and $D_R=1 \times 10^{-9}$ m²/s.

X-Scan Simulation. In this model, HeLa cell was assumed to be semielliptical shape with symmetry along z-axis. The permeability was assumed to be zero along cell membrane or arc 1 since topography was the subject of interest here. The 25 μ m tip with RG = 10 was used in the experiment and the tip was also considered symmetrical along z-axis. The tip to dish distance was maintained at 20 μ m at all times. The tip was held at diffusion controlled potential at all times over the cell or arc 1 and the model was solved in steady state solver condition with the aid of Comsol Multiphysics 4.3 software. To measure the tip current at different position over the cell, the arc 1 was moved toward left by a distance of 1 μ m out of the active simulation sub domain instead of tip moving over the arc 1.

Simulations were done first with $P = 0$ at arc 1 to determine the current at the tip for the certain fixed height of the cell. Then after that different value of P was used in the simulation to fit the experiment data. The tip current was always calculated with the tip located right above the highest point of the cell height. In this system, $P = KD / L$, which P is the velocity of ferrocyanide across cell membrane, K is a constant, D is the diffusion coefficient of ferrocyanide and L is the thickness of cell membrane. Based on the formula, the average value of P was about 6.35×10^{-6} m/s.

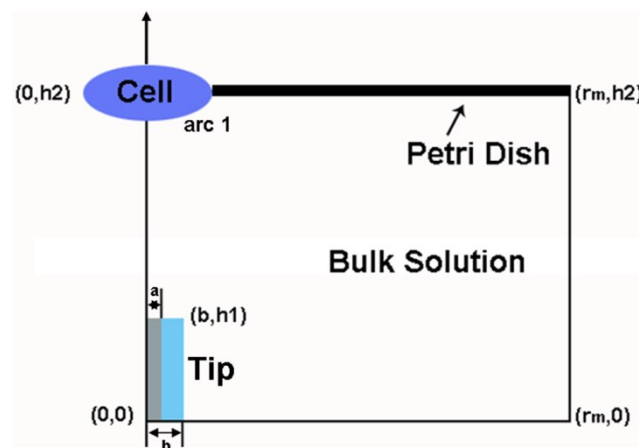


Figure S18. The schematic of simulation model.