

Supplementary Information

Monitoring the trans-membrane transporting of single fluorescent silicon nanoparticle based on force tracing technique

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Preparation of fluorescent SiNPs

SiNPs were fabricated by simple one-step hydrothermal route. Briefly, 3 ml APTES were dissolved in 10 ml deionized water. After stirring for 10 min, the mixture was transferred into a Teflon-lined stainless-steel autoclave and heated at 200 °C for 10 h. The obtained solution was filtered extensively with 0.22 μM ultrafiltration membrane to remove the large particles, and then dialyzed with a dialysis membrane in deionized water for 12 h.¹

UV-vis absorbance and FT-IR spectrum of SiNPs

UV-vis absorption spectrum of SiNPs solution is given in Fig. S1A, it shows one single broad absorption band centered at 270 nm. FT-IR spectrum of SiNPs is shown in Fig. S1B. Several distinct transmission peaks are represented in the range of 500-4000 cm^{-1} . The intense peak at around 1000 cm^{-1} could be attributed to the vibrational stretching of Si-O bond. Particularly, the peaks at around 3440 cm^{-1} and 1580 cm^{-1} are attributed to the characteristic peaks of N-H bond bending and the N-H stretching vibration, respectively. The results are consistent with previous report,¹ indicating successful synthesis of SiNPs.

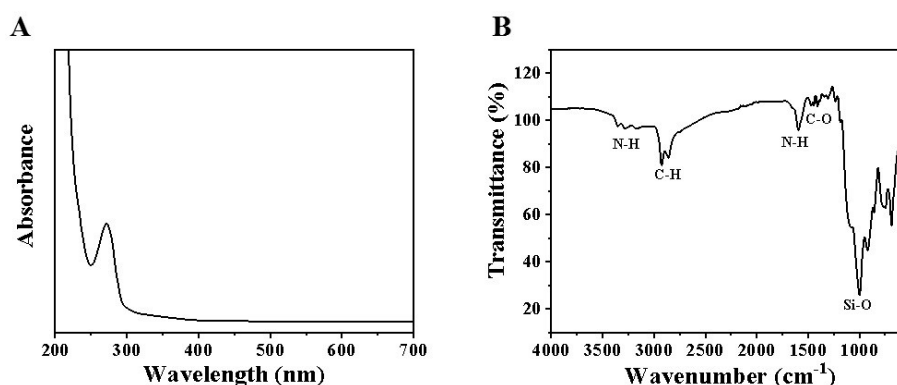


Fig. S1. The UV-vis absorbance (A) and the FT-IR spectrum of SiNPs (B).

Fluorescent spectra of SiNPs

Fig. S2 shows the fluorescent spectra of SiNPs. The highest emission intensity at 436.5 nm is obtained upon excited by 340 nm. As previous reported,¹ this absorption band is attributed to some form of oxygen deficiency in the SiO_2 network such as oxygen vacancy. Furthermore, oxygen vacancy and peroxy linkage $\equiv\text{Si-O-O-Si}\equiv$ could form self-trapped excitation in SiO_2 , which lead to fluorescent emission through recombination. The results show that SiNPs possess strong fluorescence coupled with ultrahigh photostability.

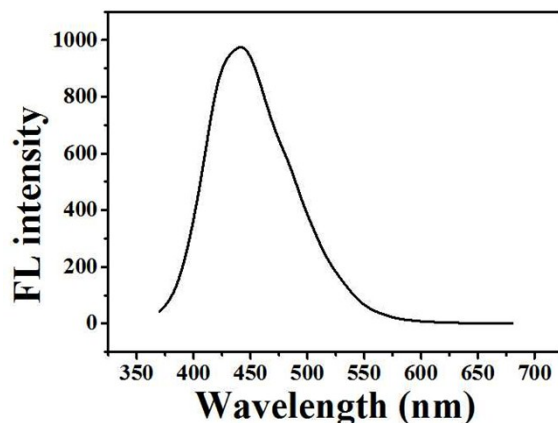


Fig. S2. The fluorescent spectroscopy of SiNPs.

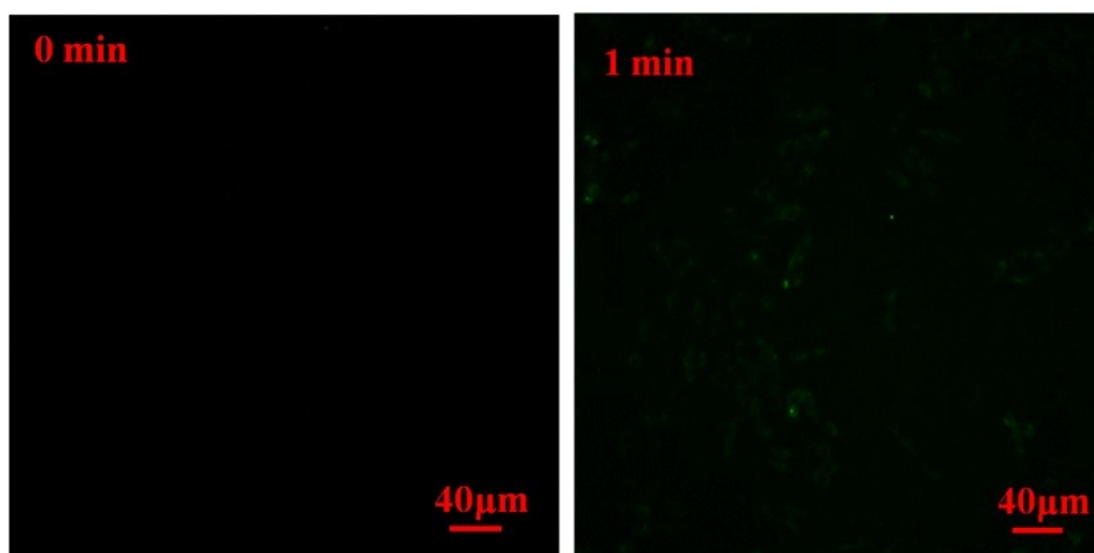


Fig. S3. The fluorescent images of Vero cells after incubation with SiNPs for 0 min and 1 min. The green dots represent the SiNPs.

Force tracing measurement

Force tracing curves were acquired by AFM 5500 (Agilent Technologies, Chandler, AZ). During experiments, the temperature was controlled at 37 °C by temperature controller 325 (Agilent Technologies, Chandler, AZ). In order to reach the contact point (Fig. S4) between the AFM tip and the cell surface, we utilized a proportional-integral (PI) control system ($P = 0.001$; $I = 0.001$; the error signal between the set point and the deflection of the cantilever is 2.0 V) to slowly move the SiNPs attached AFM tip to the contact point, and then switched off the feedback (stopping the AFM tip).² Once the trans-membrane transporting of SiNPs occurred, the cantilever deflected, and the vertical change of the cantilever was recorded by a PCI card. As a result, thousands of force curves were collected at different cells.

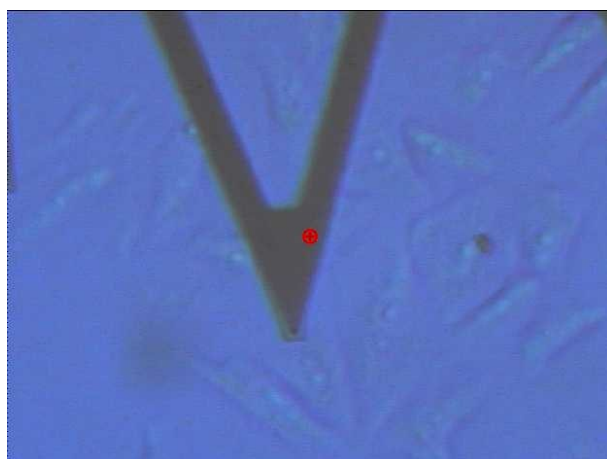


Fig. S4. AFM tip was rightly located on the top of the Vero cell monolayer with the help of a CCD camera.

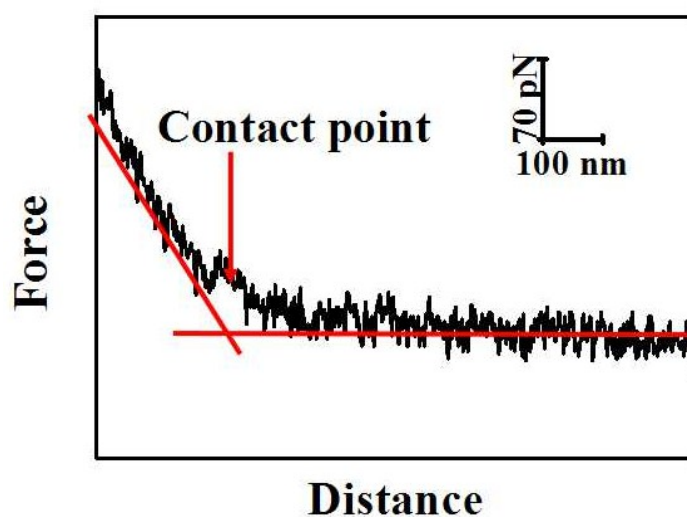


Fig. S5. The contact point between the SiNPs attached onto AFM tip and the cell surface. The contact point is the intersection of the slope (red lines) and flat part in the force-distance curve.

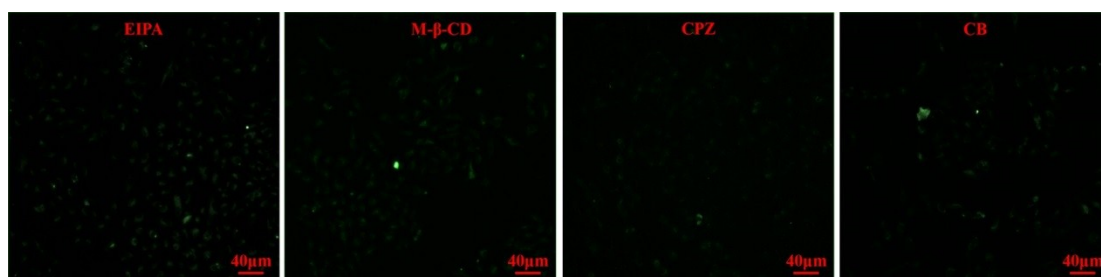


Fig. S6. Fluorescence imaging of SiNPs delivery after blocking with EIPA, M-β-CD, CPZ, and CB.

Cell culture

African green monkey kidney (Vero) cells were purchased from the Shanghai Institute of Biological Sciences (Shanghai, China), the cells were cultured in Minimum Eagle Medium (MEM, GIBCO) at 37 °C under a humidified atmosphere with 5% CO₂, supplemented with 10% fetal bovine serum (FBS, GIBCO) and 1% antibiotics (100 µg mL⁻¹ streptomycin and 100 international units mL⁻¹ penicillin). For conducting force tracing experiments, the cells were sub-cultured for 2 or 3 days to cover 75% of the petri dish. Before the force tracing tests, the adherent cells were rinsed with phosphate-buffered saline (PBS) for three times and serum-free medium one time successively to remove cell debris and unattached cells.

Modification of AFM tips with SiNPs

Firstly, The AFM tips (MSCT, Veeco, Santa Barbara, CA) were soaked in freshly prepared piranha (H₂SO₄: 30% H₂O₂, 3:1, v/v) for 1 h, then the tips were rinsed with water and ethanol before treating with O₃ (20 min). Secondly, two small plastic trays were placed inside a desiccator, flooding the desiccator with argon gas for 3 min to remove air and moisture, and then 50 µl APTES (3-aminopropyl-triethoxysilane) and 15 µl triethylamine were separately pipetted into the two trays and leaved aside for 90 min. Subsequently, the heterobifunctional PEG linker (aldehyde-PEG-NHS, FW~3962, Senso Path Technologies, Bozeman, MT) was conjugated onto the APTES-modified AFM tips in methylbenzene containing 0.5% triethylamine (v/v), and the reaction time was 2 h. Finally, the SiNPs were conjugated with the PEG linker by soaking the AFM tips in SiNPs solution with presence of 2.5 µl NaCNBH₃ for 1 h. After reaction, 5 µl ethanolamine was added into the reaction solution to passivate unreacted aldehyde groups. The modified AFM tips were washed for three times with PBS and stored in PBS at 4 °C.

Fluorescence imaging of Vero cells and SiNPs:

Fluorescence imaging of cells was executed on a fluorescence microscope (Nikon-Ti-S). Fluorescence of SiNPs was excited with a 365 nm helium-neon laser. Experiments were conducted at 25 °C. Before performing fluorescence imaging, the cells were incubated with MEM containing SiNPs (2 µg mL⁻¹) for different time, and then washed three times with PBS for imaging.

Blocking and control experiments

In the blocking experiments, Vero cells were pre-treated with cytochalasin B (CB, 2 µg mL⁻¹) for 20 min, chlorpromazine (CPZ, ultimate concentration 10 µg mL⁻¹) for 30 min, filipin (final concentration 5 µg mL⁻¹) for 30 min, methyl-β-cyclodextrin (M-β-CD, 5 mM) for 10 min, 5-(N-Ethyl-N-isopropyl) Amiloride (EIPA, 60 µM) for 1 h, nystatin (0.03 mM) for 30 min, and ferristatin (50 µM) for 2 h, respectively. Moreover,

force tracing experiments were also performed with unmodified tips (clean) and tips modified only with PEG linker to confirm that the force signal indeed from the trans-membrane transporting activity of single SiNPs rather than other contamination.

References

1. N. Xu, Y. Yuan, J.-H. Yin, X. Wang and L. Meng, *RSC Advances*, 2017, **7**, 48429-48436.
2. Y. Pan, S. Wang, Y. Shan, D. Zhang, J. Gao, M. Zhang, S. Liu, M. Cai, H. Xu and G. Li, *Small*, 2015, **11**, 2782-2788.