Supplementary Information

Recording the dynamic endocytosis of single gold nanoparticles by AFM-based Force Tracing

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Figure S1

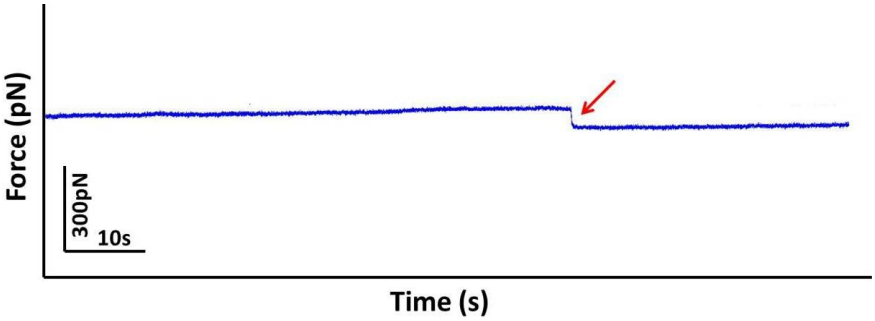

Fig.S1 Typical long time Force Tracing curve shows the endocytosis signal (pointed by the arrow) distinguished from the cellular fluctuation.

Figure S2

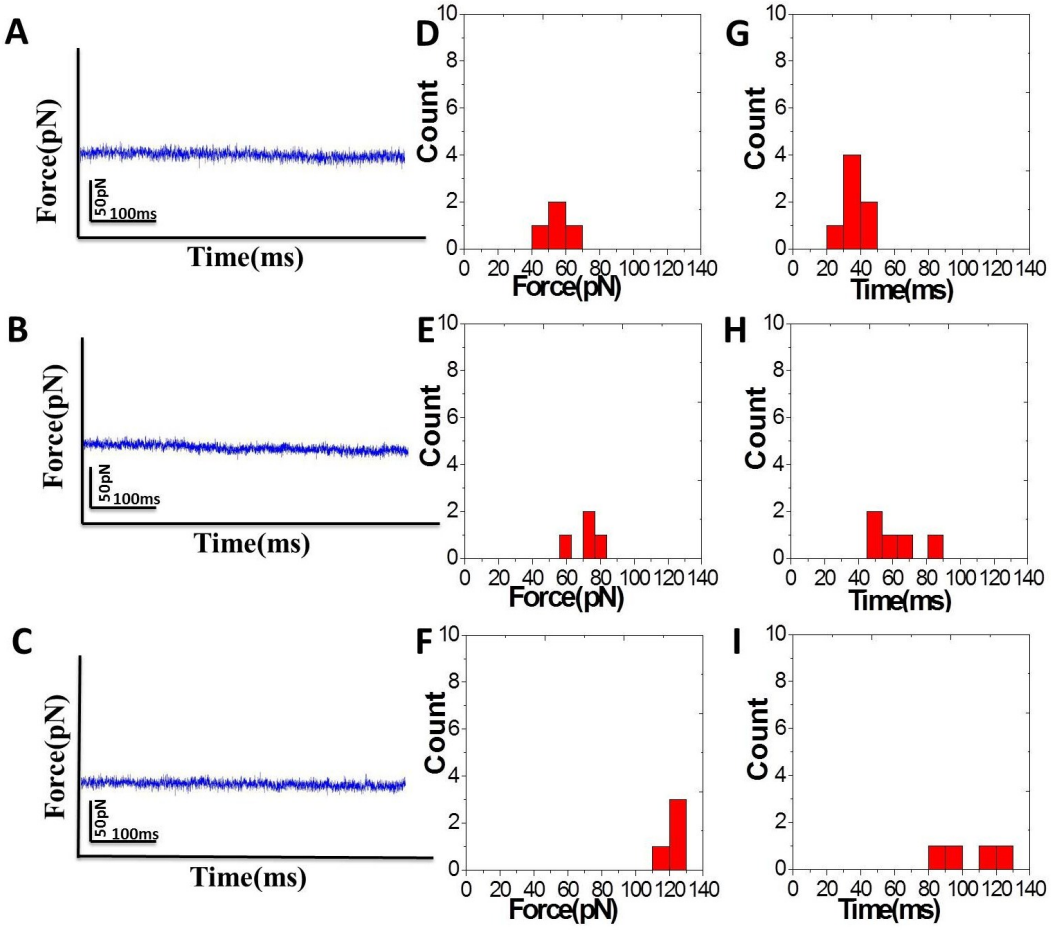

Fig.S2 Blocking the endocytosis by cytochalasin B. The Force Tracing curves of (A) 5 nm, (B) 10 nm, and (C) 20 nm Au NPs endocytosed by Vero cell after blocking with cytochalasin B. (D-F)The histograms of uptaking forces. (G-I)The corresponding histograms of actuation duration. The histograms were obtained from about 400 randomly chosen force curves after blocking with cytochalasin B.

Figure S3

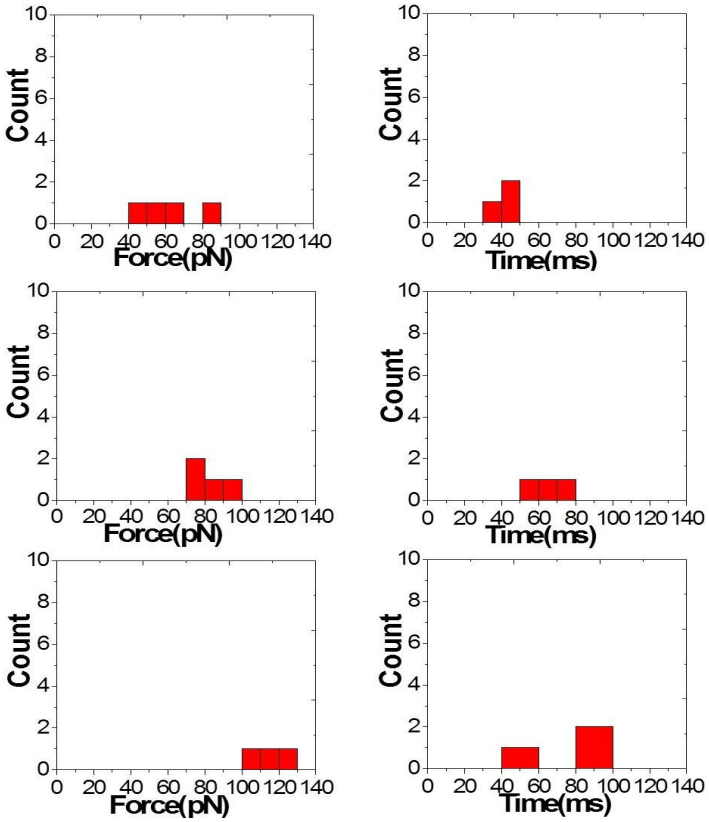

Fig. S3 Blocking the endocytosis by methyl-β-cyclodextrin. The histograms of uptaking forces for (A) 5 nm, (B) 10 nm, and (C) 20 nm Au NPs after blocking with methyl-β-cyclodextrin. (D-F) The corresponding histograms of actuation duration. The histograms were obtained from about 400 freely chosen force curves after blocking with methyl-β-cyclodextrin.

Materials and methods

Cell culture: Vero cell were obtained from the Shanghai Institutes of Biological Sciences. The cells were cultured on petri dish in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine serum albumin (Gibco BRL, Carlsbad, CA), 100μg/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Usually the cells need to be cultured for 1 or 2 days to achieve 75% confluence. Before the force spectroscopy experiments, the adherent Vero cells were washed with serum-free medium for three times to remove cellular secretions and unattached cells. During the experiments, adherent cells were supplied with serum-free medium to ensure that proteins present in serum did not affect the results of the force measurements.

Modification of AFM probes with Au NPs: AFM probes (MSCT, D-tip, Veeco, Santa Barbara, CA) were first cleaned for 30 minutes with freshly prepared Piranha solution (H_2SO_4 : 30% H_2O_2, 7 : 3, v/v), and then purged with O_3 flow for 20 minutes to further remove the organic pollution on the tips. The cleaned AFM probes were modified with APTES using vapour phase deposition method for 2 hours as previously described [1]. Subsequently, the heterobifunctional PEG linker (benzaldehyde-PEG76-NHS, FW~3962, SensoPath Technologies, Bozeman, MT) was conjugated onto the amino-modified AFM tips in chloroform containing 0.5% triethylamine (v/v). After rinsed thoroughly with chloroform and dried with argon, the AFM tips with PEG linkers were immersed into an Au NPs solution, containing 100 μL amino-capped Au NPs solution (Aladdin Letter) and 5μL 1M $NaCNBH_3$. After functionalization for 1 hour, 5μL 1M ethanomine solution was added to the solution in order to passivate the unreacted aldehyde groups. The Au NPs-modified AFM tips were washed with a large amount of phosphate buffer solution (PBS) for three times to remove noncovalently adsorbed Au NPs and stored at 4°C until use.

Force Tracing measurements: Force Tracing measurements were carried out using the AFM 5500 (Agilent Technologies, Chandler, AZ). During Force Tracing, Vero cells were kept in DMEM at 37 °C by temperature control 325 (Agilent Technologies, Chandler, AZ). We first approached the AFM tip tethered with Au NPs toward the living cell and engaged force-distance measurements to identify the contact point between the AFM tip and the cell surface. Then we utilized the 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 AFM tip to the contact point (50 nm/s). The AFM tip tethered with Au NPs would stay above the cell surface as the feedback system was switched off (stopping the AFM tip). Upon the initiation of Au NPs endocytosis, the cantilever bent downwards. The deflection of the cantilever was recorded by a 16-bit DA/AD card (PCI-6361e, National Instruments) controlled via LabVIEW software. The sampling rate is 20 kHz and the data were collected with a 100 Hz low-pass filter to eliminate high frequency noise from the electronics and environment. For statistical analysis, thousands of Force Tracing curves were recorded in different positions on the cells. In the blocking experiments, Vero cells were pretreated with cytochalasin B (1 μg/mL) and methyl-β-cyclodextrin (MβCD, 2 mM) for 30 min, respectively.The deflection sensitivity of the photo-detector and the spring constant of AFM tip were determined as previously reported [2]. The force curves were processed with

MathLab 7.9 (Math Works Inc.).

References

1. D. Lohr, R. Bash, H. Wang, J. Yodh and S. Lindsay, *Methods*, 2007, **41**, 333-341.
2. Y. Shan, X. Hao, X. Shang, M. Cai, J. Jiang, Z. Tang and H. Wang, *Chem. Commun.*, 2011, **47**, 3377-3379.