Supporting Information

Studying the dynamic mechanism of transporting single drug carrier - polyamidoamine dendrimer through cell membranes by force tracing

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Experimental section:

Cell culture: African green monkey kidney (Vero) cells were purchased from the Shanghai Institutes of Biological Sciences. The cells were maintained in a 5% CO_2 and 95% air environment at 37 °C, supplemented with Minimum Eagle Medium

(MEM, GIBCO) with 10% Fetal Bovine Serum (FBS, GIBCO), penicillin (100 international units mL^{-1}), streptomycin (100 μg mL^{-1}). The cells were sub-cultured every 2 or 3 days when the petri dish was covered by cells achieved 75% confluence. The adherent Vero cells were washed with PBS (phosphate buffer solution) for three times and serum-free medium one time to remove cell debris and unattached cells before used in the force tracing experiments.

Modification of AFM probes with G5-PAMAM NPs: Firstly, AFM probes (MSCT, Veeco, Santa Barbara, CA) were soaking for 1 h in freshly prepared Piranha solution (H_2SO_4: 30% H_2O_2, 3:1, v/v). Secondly, probes were purged with O_3 flow for 20 min to further remove the organic pollution on the tips after cleaning with water, ethanol, and dried by argon flow, then dried for 2 h in dryer. Subsequently, probes were modified by 3-aminopropyltriethoxysilane (APTES) using vapor phase deposition method for 80 min as previously described[1], combined with the heterobifunctional PEG (benzaldehyde-PEG76-NHS, FW~3962, SensoPath Technologies, Bozeman, MT 1mg mL^{-1}) in methylbenzene with the absence of 0.5% triethylamine (v/v) for 2 h. Then, the probes were conjugated with G5-PAMAM (polyamidoamine, 5 mg mL^{-1}) in PBS solution with the absence of 0.01 M $NaCNBH_3$ for 1 h after drying by argon flow. To deactivate the aldehyde groups that didn't react, 5 μL 1 M ethanolamine was added into the PBS solution reacted for 10 min after that. Finally, the probes were washed by PBS solution for three times and stored at 4 °C until use.

The technology of single molecular force tracing based on AFM: The technology was invented based on AFM 5500 (Agilent Technologies, Chandler, AZ). The

experiment was carried out at 37 °C by temperature control 325 (Agilent Technologies, Chandler, AZ) after the petri dish was washed by PBS as mentioned above, and then 2 mL MEM was added into petri dish. The sensitivity of photo-detector and the spring constant of AFM tip were set right as reported previously[2]. To perform the force tracing experiments, AFM tip modified with G5-PAMAM was right located onto the dense Vero cells by CCD camera, which could ensure that every experiment was accomplished on the surface of cells.

In order to find the contact point between AFM tip and living cell surface, a modified AFM tip was approached and engaged through force-distance measurements. To determine the contact point, we moved the AFM tip to the contact point slowly, while 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) was open. Then the feedback system was turned off when the G5-PAMAM attached AFM tip was just above the cell surface. The cantilever would bend downwards because of the endocytosis of G5-PAMAM by Vero cells, and the deflection of the cantilever was recorded by a 16-bit DA/AD card (PCI-6361e, National Instruments), which was controlled by LabVIEW software. During the experiment, thousands of force curves were collected at different zones of different cells and analyzed by MATLAB 7.9 (Math Works Inc, Natick, MA, USA).

Blocking and control experiments: In blocking experiments, Vero cells were incubated with cytochalasin B (final concentration 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, and nystatin (0.03 mM) for 30 min, respectively. In control experiments, without any modified tip and tip modified with PEG linker were used to perform force tracing experiments, to confirm that the force signals really resulted from the cellular uptake of G5-PAMAM rather than other events.

The fluorescence staining of cells and G5-PAMAM: The cell membranes were stained with lipophilic dyes. The Vero cells were incubated in MEM with DiO for 30 min at room temperature, then washed by PBS for three times to remove extra dyes and added 1 mL PBS to proceed fluorescence experiment. For labelling G5-PAMAM, Alexa532 was incubated with 5 mg mL^{-1} nanoparticles at room temperature for 3 h with hard vortexing on the purpose of mixing uniformity. To remove the unbound Alexa532, the nanoparticles mixture were purified through G-25 chromatographic column and stored at 4 °C before examined under fluorescence microscope.

Fluorescence imaging of Vero cell and G5-PAMAM: The fluorescence imaging was carried out using a Leica TCS SP2 confocal Microscope. Alexa532 labeled G5-PAMAM nanoparticles was excited with a 543 nm helium-neon laser, while the DiO-labeled membranes were excited with a 488 nm Ar-Kr laser. The fluorescence images were collected with a NA=1.40 100×oil immersion objective. Experiments were conducted at 25 °C. Data was collected using a photomultiplier tube (PMT) and processed with Leica TCS software. The fluorescence intensity was analyzed by MATLAB.

Locating the contact point:

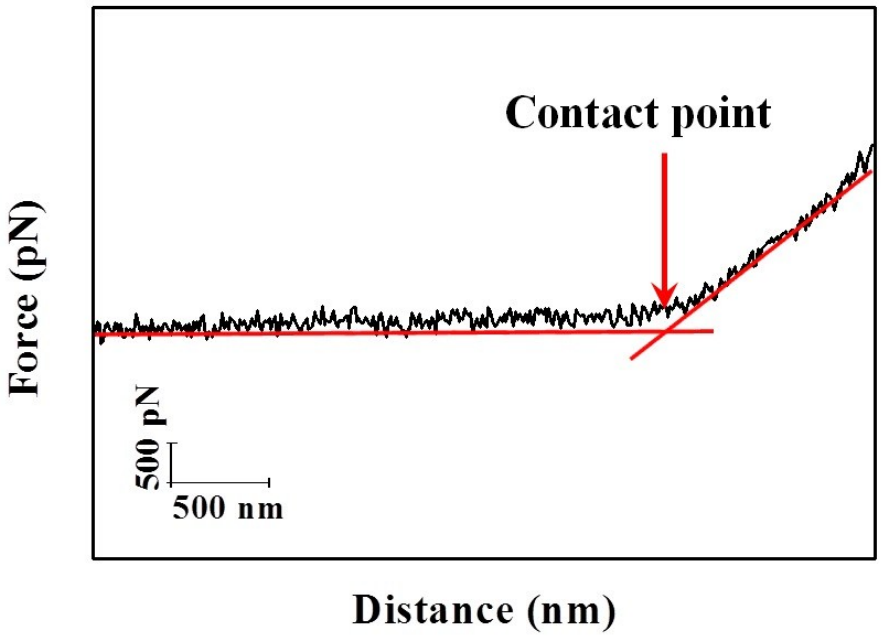

Fig. S1. Engaging the G5-PAMAM modified AFM tip to the cell surface and defining the point at which the AFM tip first contacts the cell, shown as the red arrow. The contact point is the intersection of flat part and slope (red lines) in the force-distance curve.

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.