Electronic Supplementary Information

Single Molecular Localizations of Voltage-Gated Sodium Channel $Na_V1.5$ on the Surfaces of Normal and Cancer Breast Cells†

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1. Control experiments: Single molecular recognition imaging of $Na_V1.5$ on the surface of normal breast cells by the bare tips or PEG modified tips

The recognition images on the surfaces of Hs578Bst cells acquired by the bare tips or PEG modified tips are shown in Fig. S1A and S1B, respevtively. There are no recognition signals on both images, which indicates that the recognition signals in Figure 2 and Figure 3 are indeed from the specific interactions between the $Na_V1.5$ on the surfaces of cells and anti-$Na_V1.5$ antibody modified on the tips.

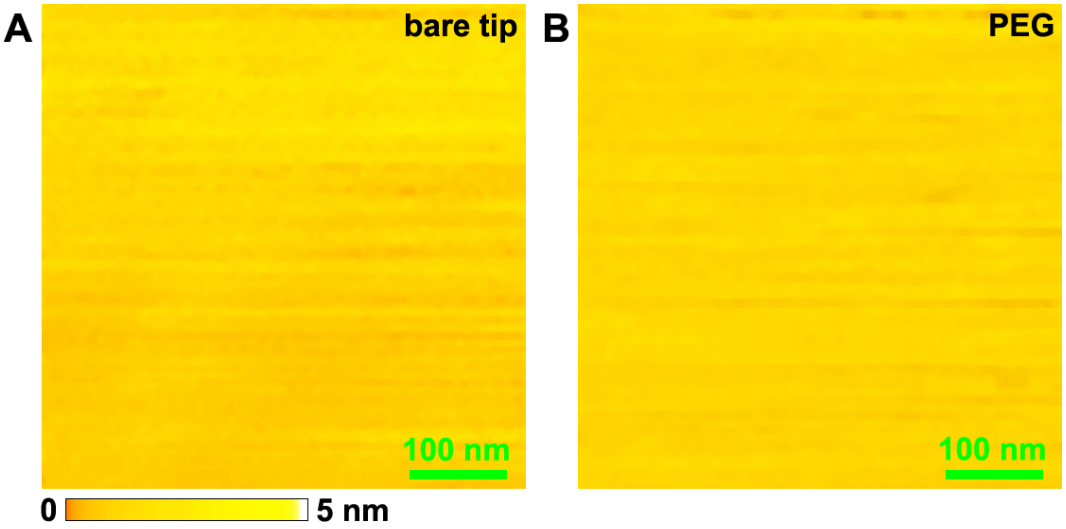

Fig. S1. The recognition images on the surfaces of Hs578Bst cells performed by the bare tips or PEG modified tips.

2. Experimental sections

Cell culture

Hs578Bst and MDA-MB-231 cells were chosen as the representative normal and cancer breast cells, and purchased from Procell Life Science and Technology Co., Ltd (Wuhan, China). All these cells were cultured in Dulbecco's minimum essential medium (DMEM, Biological Industries, Shanghai, China) with 10% fetal bovine serum (Biological Industries, Shanghai, China), 100 μg/mL streptomycin (Solarbio life sciences, Beijing, China) and 100 U/mL penicillin (Solarbio life sciences, Beijing, China). Cells were cultured in a humidified atmosphere with 5% CO_2 at 37°C in incubator, and were grown as monolayer for use.

Fluorescence staining and fluorescence microscopy

When the cells were cultured well in the petri dish, they were washed three times by phosphate buffer saline (PBS, Corning). Then the cells were fixed with 4% paraformaldehyde for 20 min, and washed with PBS for three times. The nonspecific binding sites on cells were blocked by 2% bovine serum albumin (Solarbio life sciences, Beijing, China) for 2 h. The cells were reacted with anti-$Na_v1.5$ polyclonal antibody (Abcam, Shanghai, China) at 4°C overnight. Then reacted with donkey anti-goat IgG (Alexa Fluor 488, Abcam, Shanghai, China) for 1 h in darkness. Before imaging, the cells were washed with PBS for three times to remove the unreacted dye.

The fluorescence images were obtained with the laser scanning confocal microscopy LSM880 (Carl Zeiss, Shanghai, China). The Alexa Fluor 488 was excited by the laser with the wavelength of 488 nm, and the emission fluorescence (wavelength of 515 nm) was collected by the 20× or 63× objective. All the data were processed by software Zen 2.3 (Carl Zeiss, Shanghai, China).

Functionalization of the AFM tips with anti-$Na_V1.5$ antibody

The functionalization procedures were similar as described previously [1]. Briefly, anti-$Na_V1.5$ antibodies were reacted with N-succinimidyl 3-(acetylthio) propionate (SATP, Sigma-Aldrich, Shanghai, China). The cantilevers were cleaned in the O_3 atmosphere in ultraviolet radiation cleaner for 20 min to get rid of the organic contamination. Then the cantilevers were vapor treated with aminopropyltriethoxysilane (APTES, 99%, Sigma-Aldrich, Shanghai, China), and reacted with polyethylene glycol (PEG) crosslinkers (9.8 nm in length, MaL-PEG2000-NHS, JenKem Technology Co., Ltd., Beijing, China) in triethylamine (Sigma-Aldrich, Shanghai, China) and $CHCl_3$ (Richjoint Chemical, Shanghai, China). Then the cantilevers were immersed in 100 μg/mL anti-$Na_V1.5$ antibody with $NaCNBH_3$ (Sigma-Aldrich, Shanghai, China) as catalyst. In the last, 1 M ethanolamine (Sigma-Aldrich, Shanghai, China) was added to passivate the unreacted aldehyde groups. Then the modified tips were rinsed with PBS for two times and stored in PBS at 4°C until use.

Atomic force microscopy

All the experiments were performed with the JPK NanoWizard 4XP BioScience AFM (Bruker Corporation, Santa Barbara, California, USA). Single molecular recognition imaging was performed by the QI advanced mode, and was carried out with anti-$Na_V1.5$ antibody modified tips in buffer solutions at room temperature. The probes were MLCT-Bio-C (Bruker Corporation, Santa Barbara, California, USA), and the main parameters were (nominal): resonance frequency 7 kHz, spring constant 0.01 N/m. The scanning rate is 1 Hz. The recognition signals were revealed at the 75% cut-off of the background. Blocking experiments were performed by the addition of the 100 μg/mL anti-$Na_V1.5$ antibody into the AFM sample cell.

Force spectroscopy was operated in the contact force spectroscopy mode. The deflection sensitivity of the photo-detector was determined by the slope of the force curves captured on the surfaces of cleaning silicon wafer. The actual spring constants of the cantilevers were measured by the thermal noise method in air as described previously [2]. Thousands of force curves were obtained on various positions of different cells. Blocking experiments were performed by the addition of 100 μg/mL anti-$Na_V1.5$ antibody into the AFM sample cell.

All the AFM images and force curves were processed by the software JPK SPM Data Processing 7.0 (Bruker Corporation, Santa Barbara, California, USA).

1. J. G. Jiang, X. Hao, M. J. Cai, Y. P. Shan, X. Shang, Z. Y. Tang and H. D. Wang, *Nano Lett.*, 2009, **9**, 4489-4493.
2. H. J. Butt and M. Jaschke, *Nanotechnology*, 1995, **6**, 1-7.