Support information

Single molecular recognition force spectroscope study of DNA aptamer with target epithelial cell adhesion molecule

Experiments and Materials

Materials: Recombinant Human EpCAM protein was purchased from ACROBiosystems (China). Aptamer SYL3C (5'-NH_2-TTTTTTCAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCATGG GGG GTT GGC CTG-3') was customized from Sangon Biological Engineering Technology & Services Co., Ltd. (China).NHS-PEG_{18}-acetal was purchased from Prof. Hermann J. Gruber (Johannes Kepler University, Austria). Silicon wafers were obtained from Shengxu Electronic Technology Co., Ltd (China). 3-aminopropyltriethoxysilane (APTES), triethylamine and ethanolamine were purchased from Sigma-Aldrich (USA). $NaCNBH_3$ and Citric acid (anhydrous, 99.5+%) were supplied by Alfa Aesar (USA). Other reagents used in all experiments were of analytical grade. Milli-Q-purified water (18.2 MΩ) was used for all solution preparations.

Modification of the AFM tips with aptamer SYL3C: The aptamer SYL3C was covalently attached to AFM tip (MSCT, Si_3N_4, Veeco, CA) via the PEG cross-linker (NHS-PEG_{18}-acetal). In brief, the AFM tips were cleaned with ethanol and chloroform three times, and dried under nitrogen stream. Using a vapor deposition method, the cleaned AFM tips were first amino-functionalized by a 2 hours incubation in a desiccator with 15 μL triethylamine and 50 μL APTES. Then, the tips were incubated in chloroform containing a 2 mg mL^{-1} PEG cross-linker and 0.5% (v/v) of triethylamine 2 hours to couple the PEG onto AFM tips. In the third step, the acetal functions were converted into aldehyde groups by immersing the tips in 1% citric acid (pH 2.2) for 10 minutes and then washing with Milli-Q water. The coupling of SYL3C was accomplished by immersing AFM tips in 100 μg mL^{-1} SYL3C in PBS buffer (137 mM NaCl, 2 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, and pH 7.4) containing 20 mM of freshly prepared $NaCNBH_3$ solution about 1 h at room temperature. Finally, a final concentration of 25 mM ethanolamine was added to passivate the unoccupied aldehyde groups. The prepared AFM tips were washed with PBS buffer and stored in PBS buffer at 4℃.

Silicon substrate surface functionalization: The general process for modifying silicon substrate with EpCAM was similar to the tip functionalization. Briefly after cleaning with ethanol and chloroform, the cleaned silicon substrates were first amino-functionalized using a vapor deposition method by incubating with 15 μL triethylamine and 50 μL APTES for 2 hours in a desiccator. Then the amino functionalized silicon substrates were incubated in 2 mg/ml NHS-PEG_{18}-acetal in chloroform containing 0.5% (v/v) of triethylamine for 2 hour. In the third step, the acetal functions were converted into aldehyde groups by immersing the tips in 1% citric acid (pH 2.2) for 10 min and then washing with Milli-Q water. The functionalized silicon substrates were incubated in 100 μgmL^{-1} EpCAM protein solution containing a final concentration of 20 mM freshly prepared $NaCNBH_3$ solution about 1 h at room temperature. Finally, a final concentration of 25 mM ethanolamine was added to passivate the unoccupied aldehyde groups. The silicon substrates functionalized with EpCAM were washed with PBS buffer and stored in PBS buffer at 4℃.

SMFS measurements: All SMFS measurements were performed on a PicoPSM 5500 (Agilent Technologies, MA, USA) in PBS buffer at room temperature using MSCT tips (Si_3N_4, Veeco, CA). Thousands force–distance curves were collected for a particular loading rate. The experiment for each loading rate was performed with a same tip at randomly selected three to six locations on each silicon substrate. The experiment was repeated several times on different samples. Force distributions were presented as empirical probability density functions (pdf). Loading rates r were calculated by the equation $r = v \times k_{eff}$, with v being the pulling velocity and k_{eff} being the effective spring constant. The spring constants of the cantilevers were determined by the thermal-noise method. Analysis of force−distance curves was performed with Matlab 7.1 (Math works, MA).

AFM topographical images: Topographical images were acquired on a multimode 8 AFM system (Veeco, U.S.A) operated in tapping mode using SNL-10 tips (Si_3N_4, Veeco, CA). All images were taken in PBS at room temperature. The value of the setpoint was regulated to minimize the possible damage to the sample and to maintain the stable image quality at the same time. All AFM images were height images and were raw data except for those flattened using a standared algorithm within the Nanoscope software to remove artificial height offsets between consecutive scan lines of the raw images.

Block experiment: The same samples and the same tip were used to perform the block

experiment. The EpCAM functionalized silicon substrate was incubated in 100 μg mL^{-1} aptamer solution for 1 hour. After the unoccupied aptamers were flushed with PBS binding buffer, SMFS measurement was used to collect force–distance curves in PBS buffer.

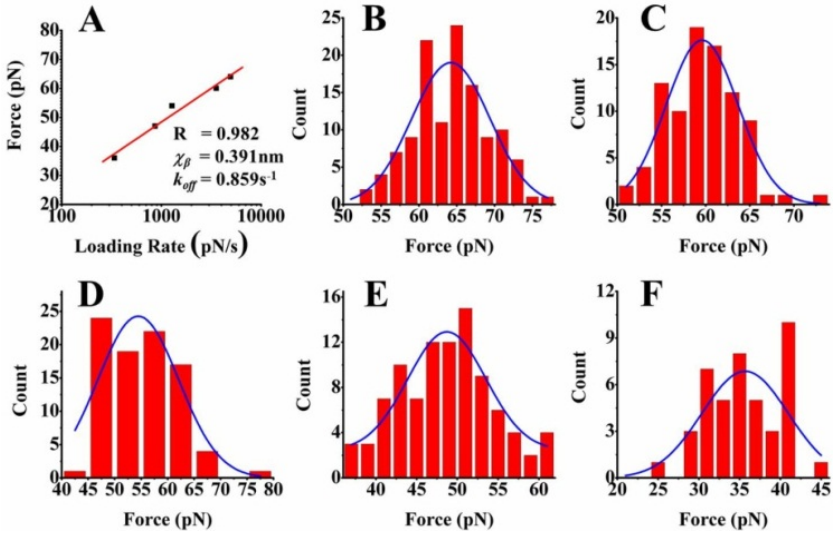

Fig. S1 SMFS study of the unbinding force distribution of the SYL3C and EpCAM at the present of 5mM Mg^{2+}. (A) The dependence of the unbinding force (f_u) on the loading rates (r). (B–F) The f_u distribution at different loading rates: 4.950 nN s^{-1}, 3.550 nN s^{-1}, 1.280 nN s^{-1}, 0.864 nN s^{-1}, and 0.338 nN s^{-1}, respectively.