Supporting Information

Mapping the Interaction Sites of Mucin 1 and DNA Aptamer by Atomic Force Microscopy

Materials. Recombinant Human MUC1 was purchased from ACROBiosystems (China). DNA aptamer (5'-NH_2-TTTTTTGCAGTTGATCCTTTGGCCTGG-3') [26] of MUC1 was purchased from Sangon Biological Engineering Technology & Services Co., Ltd. (China). NHS-PEG_{18}-acetal was purchased from Prof. Hermann J. Gruber (Johannes Kepler University, Austria). 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). PBS buffer (pH 7.4) combined 137 mM NaCl, 2 mM KCl, 8 mM Na_2HPO_4, and 1.5 mM KH_2PO_4. Adsorption buffer (pH 7.8) for MUCI combined 300 mM KCl, 5 mM $MgCl_2$ and 10 mM Tris-HCl. Other reagents used in all experiments were of analytical grade. Milli-Q-purified water (18.2 MΩ) was used for all solution preparations.

Functionalization of the AFM tips with aptamer. AFM tips **(**Veeco, U.S.A) were functionalized with aptamer through a heterobifunctional cross-linker as described before[1, 2-5]. The MSCT-E AFM tips (with a spring constants of ≈0.1 Nm^{-1}) was used for peak-force tapping measurements, and MSCT-B AFM tips (with nominal spring

constants of≈0.02 N m^{-1}) was used for SMFS measurements. Breifly, AFM tips were cleaned with ethanol and Milli-Q-purified water three times, and dried with filtered argon stream. Using the vapor deposition method[3], the cleaned AFM tips were amino-functionalized by incubated in a desiccator with 15 μL triethylamine and 50 μL APTES for 2 hours. For the linker attachment, the tips were immersed in solution of chloroform containing a 2 mg mL^{-1} NHS-PEG_{18}-acetal and 0.5% (v/v) of triethylamine 2 hours[4, 5]. This linking mechanism has been shown to provide sufficient mobility and flexibility to biological samples to rotate and orient themselves for binding[1]. In the third step, the tips were immersed in 1% citric acid (pH 2.2) for 10 minutes to convert the acetal functions into aldehyde groups and then washed with Milli-Q water. The aptamer was coupled to the tips by immersing AFM tips in 100 μL of 100 μg mL^{-1} aptamer solution (dissolved in PBS buffer containing 20 mM of freshly prepared $NaCNBH_3$) for 1 hour at room temperature. Finally, the unoccupied aldehyde groups were passivated by adding a final concentration of 25 mM ethanolamine. This method of functionalization is relatively simple, mild, and the conformation of the biological samples usually does not alter[2, 3, 5].The prepared AFM tips were washed with PBS buffer three times and stored in PBS buffer at 4℃.

Peak-force tapping measurements. 25 μL of 0.5 μg mL^{-1} MUC1 solution dissolved in adsorption buffer was adsorbed to freshly cleaved mica for 1 hour. High-electrolyte buffers[6, 7] (300 mM KCl) were preferred to adsorb MUC1 to the negatively charged mica surface. Divalent ions (Mg^{2+})[6] were added to more efficiently compensate the electrostatic repulsion between mica and MUC1. Then the sample

was rinsed the samples with the PBS buffer five times. The functionalized tips were used to locate MUC1 adsorbed on mica. It was operated in peak-force quantitative nanomechanical mapping mode (PeakForce QNM) on multimode 8 AFM systems (Veeco, U.S.A). All experiments were done in PBS buffer solution at room temperature. AFM images were recorded at 256 pixels×256 pixels with a driving frequency of 0.25 kHz, and with an amplitude of 100 nm, and an peak force setpoint of 300~350 pN. To confirm the specifity of the interaction between aptamer and MUC1, a nonfunctionalized AFM tip was used to detect MUC1 on mica. The sample was imaged at the same parameter setting. Cantilever spring constant and sensitivity were calibrated by the thermal-noise method[8]. Data analysis was performed with Nanoscope Analysist1.5 (Bruker AXS Corporation) and Matlab 2015b (Math works, MA).

SMFS measurements. For the SMFS measurements, MUC1 was immobilized on silicon substrate. The general process for modifying silicon substrate with MUC1 was similar to the tip functionalization[9]. NHS-PEG_{18}-acetal was attached to the silicon substrates. Then the functionalized silicon substrates were incubated in 100 μL of 100 μg mL^{-1} MUC1 solution containing a final concentration of 20 mM freshly prepared $NaCNBH_3$ solution for 1 hour at room temperature. Finally, a final concentration of 25 mM ethanolamine was added to passivate the unoccupied aldehyde groups. The MUC1 samples were washed with PBS buffer and stored in PBS buffer at 4℃. All SMFS measurements were performed on Multimode8 AFM system (Veeco, U.S.A) in PBS buffer at room temperature. Force-distance curves were obtained by repeatedly

approaching and subsequently retracting the aptamer-functionalized tip to MUCI samples. A schematic drawing of a typical force–distance cycle was presented in Fig. 1B. At the beginning of the approach (green line) the cantilever deflection remained zero (position 1 to 2). Once the cantilever contacted the sample surface it started to bend until a force setpoint was reached (position 2 to 3). Subsequently, the tip retracted from the sample surface (red line).During retraction, if a specific interaction event of the aptamer and MUC1 occurred, the cantilever was bent downward in a nonlinear way (position 3 to 4). At a critical force, the rupture force, the binding of the aptamer and MUC1 broke and the tip jumped back to resting position with no deflection of the cantilever (position 4 to 5). The nonlinear delay preceding the jump was caused by the length of the extended PEG_{18} linker. Those unbinding events which were characterized by a nonlinear delay preceding the jump were considered to be specific. During this cycle, the cantilever deflection (x) was continuously measured and converted into a force (f) according to Hook's law (F= kx, where k is the cantilever spring constant). The experiment for each loading rate was performed with a same tip at randomly selected three to six locations on each sample. Thousands force–distance curves were collected for each particular loading rate. 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 calibrated thermal-noise method[8]. For each loading rates, a Gaussian of unitary area with the width was positioned and then simply summed up to give the probability density functions (pdf) as described earlier[10]. Probability density functions displayed

the most probable rupture force peak. The experiment was repeated several times on different samples. For the block experiment, the MUC1 sample was incubated in a concentration of 100 μg mL^{-1} free aptamer solution for 1 hour. After the unoccupied aptamers were flushed with PBS binding buffer, SMFS measurement with the aptamer functionalized AFM tips was used to collect force–distance curves in PBS buffer. Analysis of force−distance curves was performed with Nanoscope Analysist1.5 and Matlab 2015b.

Block experiment in SMFS.

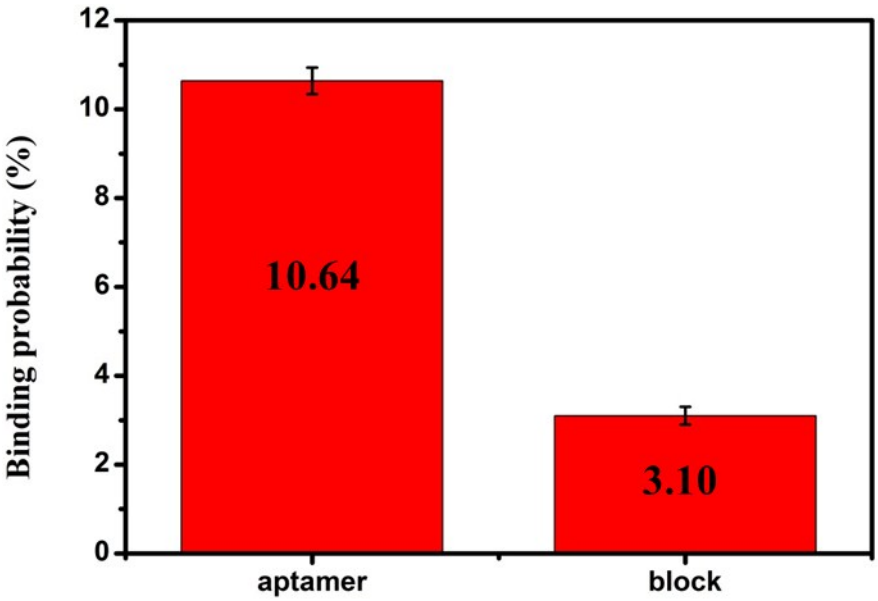

Figure.S1 The histogram of binding probability of aptamer and MUC1 before and after incubating the MUC1 samples in free aptamer.

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