Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Single Antibody-Antigen Interactions Monitored via Transient Ionic Current with Nanopore Sensors

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EXPERIMENTAL SECTION

1. Materials

All reagents were analytical grade and used as received without purification. Mercaptoacetic acid, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Sodium phosphate monobasic (NaH_2PO_4, 99%), sodium phosphate dibasic (Na_2HPO_4, 99%) and potassium chloride (KCl, 99%) were obtained from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Ultrapure water (18.2 MΩ) from Millipore system (EMD Millipore, Billerica, MA, U.S.A.) was used to prepare solutions in the experiment. The electrodes used in the experiment was sliver wire (0.25 mm, Alfa Aesar Co., Ward Hill, MA, U.S.A.). The AFP antigen and the antibody were provided by Prof. Huan-Xing Han (Translational Medical Center, Changzheng Hospital, The Second Military Medical University, P. R. China).

2. The fabrication and modification of quartz nanopipettes with specific antibody

Briefly, the quartz capillaries (Sutter Instrument, outer diameter 1.0 mm, inner diameter 0.5 mm) were cleaned, dried and pulled by P-2000 laser puller (Sutter Instrument Co., Novato, CA, U.S.A.). The parameters for pulling the nanopipette: Heat 650, Fil 3, Vel 35, Del 170, Pul 205. The quartz nanopore was thus obtained and then followed by the gold coated and modification of specific antibody which was described in the main text in detail. Both the bare quartz nanopore and gold coated nanopore were characterized by SEM using the Zeiss Ultra Plus scanning electron microscope.

To confirm the successful modification of antibody on the gold coated nanopipette, time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to characterize the modified antibody on the gold surface. Here, we used the same modification process as the modification of gold coated nanopipette to functionalize anti-AFP on the gold coated ITO surface. Then, the Mass spectra were obtained after washing the functionalized ITO with deionized water. We selected several characteristic peaks of amino acids for the SIMS imaging (Figure S4). As demonstrated, there were higher intensities in the antibody modified ITO compared with the control experiment. SIMS images of these species reveal a uniform coverage of the antibody on the gold layer according to our modification method.

3. Nanopipette experiment and data analysis

The gold coated quartz nanopore (or antibody functionalized nanopore) was filled with 1M KCl solution by using a microloader and then centrifuged to remove the remaining air in the tip. The working electrode was Ag/AgCl electrode which was embedded inside the nanopore; the counter electrode was also Ag/AgCl electrode which was immersed in the solution outside the nanopore. The experiments were performed at room temperature and the protein solutions were all freshly prepared before each use. Both the current-voltage (I-V) curves and current traces were amplified and collected by a current amplifier (Axon 200b, Axon Instrument, Forest City, USA) with a 5-kHz low-pass Bessel filter. A DigiData 1550A converter and a PC running PClamp 10.4 (Axon Instrument, Forest City, USA) were used to measure the current at a sampling frequency of 100 KHz. The raw data was analyzed by using our self-developed software

and OriginLab 8.5. Considering the signal-to-noise ratio, here we set the threshold of 50 pA for extracting the duration and current amplitude from each event.

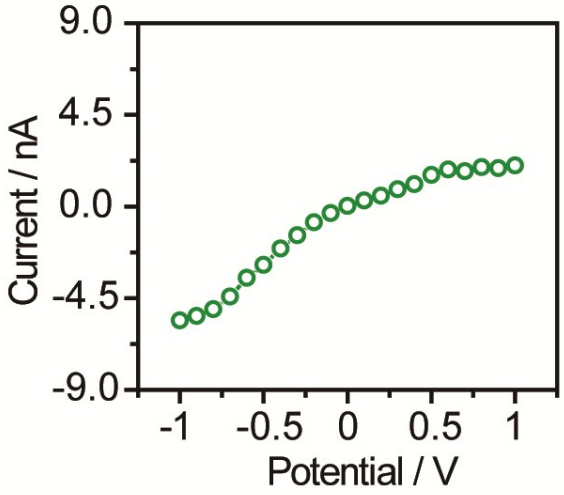

Figure S1. The I-V response of the mercaptoacetic acid functionalized quartz nanopore measured in 1 M KCl.

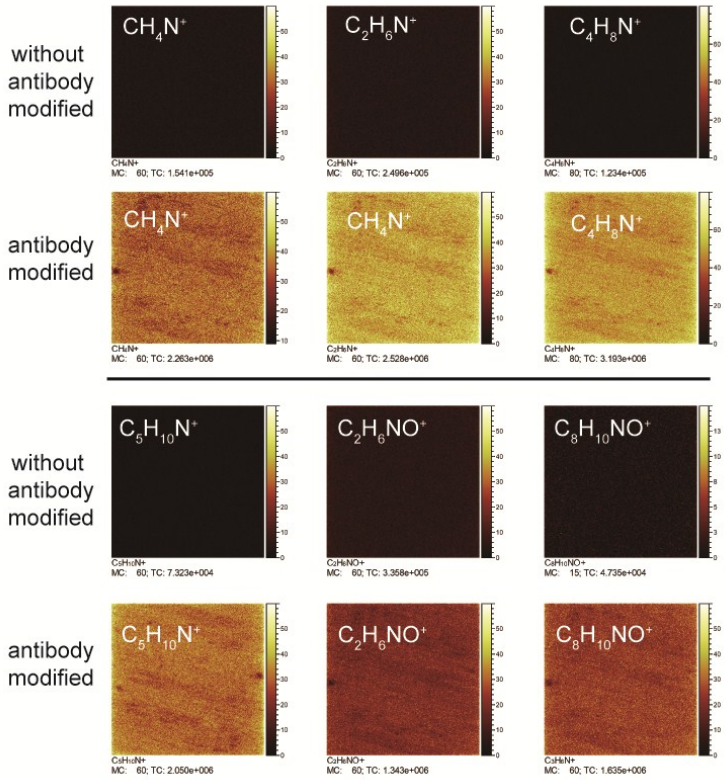

Figure S2. Positive ion ToF-SIMS images of the gold layer without (upper) and with the antibody modified (bottom). The selected species are CH_4N^+, $C_2H_6N^+$, $C_4H_6N^+$, $C_5H_{10}N^+$, $C_2H_6NO^+$ and $C_8H_{10}NO^+$, respectively.

Figure S3. The raw current trace from antibody-modified nanopore in 1 M KCl.

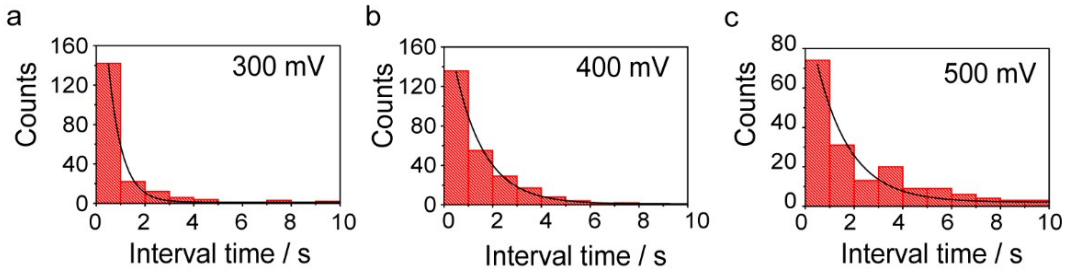

Figure S4. Interval times obtained at different voltages using antibody functionalzied quartz nanopore.