The Supporting Information for

Direct Real-Time Detection of Single Proteins Using Silicon Nanowire-based Electrical Circuits

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I. Device fabrication and surface functionalization

The nanowire growth procedure is similar to those reported in the previous studies.[1-4] Silicon wafers with a 1000 nm-thick thermal oxide layer were used as growth substrates and gold nanoparticles with an average diameter of ~20 nm (Ted Pella) were used as catalysts. Boron-doped p-type SiNWs were synthesized at 465 ºC for about 25 min, using 2.5 sccm disilane (Matheson Gas Products, 99.998% Purity) as the reactant source, 0.11 sccm diborane (100 ppm, diluted in H_2) as the p-type dopant with a B/Si ratio of 1/100000, and 7.5 sccm H_2 as the carrier gas. Both scanning electron microscopic (SEM) and transmission electron microscopic (TEM) characterizations reveal that uniform high-quality SiNWs have the nice core/shell structure with the single-crystalline core sheathed with averagely a ~5 nm-thick layer of amorphous SiO_2 (Fig. S1).

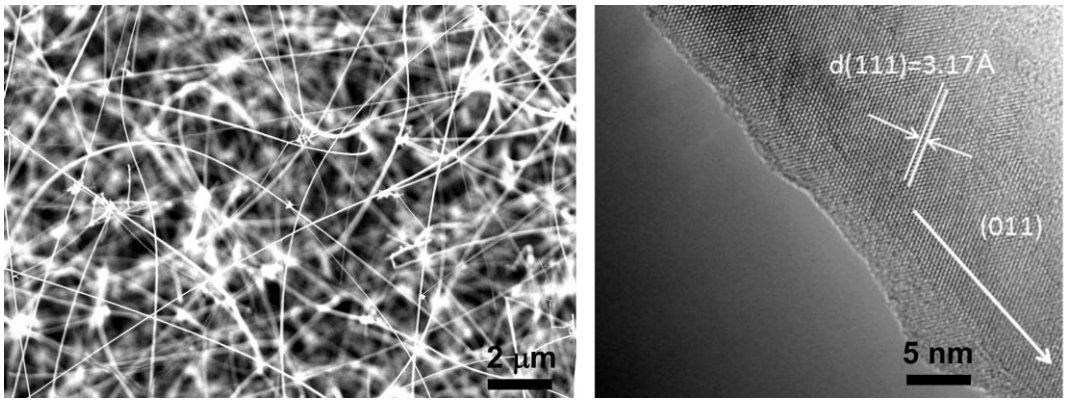

Fig. S1. (A) SEM image of pristine SiNWs grown by a standard Au-catalyzed vapor–liquid–solid method. (B) HRTEM picture of individual SiNWs.

After SiNWs growth, we directly functionalized the surface of pristine SiNWs with a gas-phase (3-(2-aminoethylamino) propyltrimethoxysilane (N-APTMS, Sigma-Aldrich, 99%) in a vacuum desiccator for ~8 h at 120 ºC. This operation also kept the

original upright growth morphology of SiNWs so that we could easily transfer the functional SiNWs to the clean doped silicon wafers with circa 1000 nm of thermally grown SiO_2 on the surface and well-aligned by mechano-sliding.[5] After transferring amino SiNWs to the defined area designed for electrode patterning, we proceeded a standard photolithographic process (UV Exposure machine, BG¬401A, China Electronics Technology Group Corporation) to open the pattern window. Then, 8 nm Cr followed by 80 nm Au were deposited through thermal evaporation (ZHD-300, Beijing Technol Science) to form metal electrodes. Before photoresist lift-off, another 50 nm-thick SiO_2 protective layer was deposited through electron beam thermal evaporation (TEMD-600, Beijing Technol Science) in order to passivate the contact interfaces. After SiO_2 deposition, we washed silicon wafers by copious acetone and achieved SiNW FET arrays as shown in Fig. S2.

After device fabrication, we put the devices into an ethanol solution of PDITC (1 mg/ml, Sigma-Aldrich, 99%) at 40 ^oC for ~2 h to attach PDITC on the surface of amino SiNWs through thiourea formation, leaving another –NCS group of PDITC for the following modification. After PDITC treatment, the devices were washed by ethanol and deionized water. Then, a drop of an aqueous solution containing 5 mM AB-NTA (Sigma-Aldrich, 99%) was placed on top of the devices for ~2 h. This treatment initiated the reaction between AB-NTA and the free –NCS group of PDITC. After washing with deionized water for 2 min, the devices were immersed into an aqueous solution of $NiCl_2$ (5 mM, Sigma-Aldrich, 99.99%) for 10 min to form functional Ni-NTA end groups,

which is ready for protein detection.

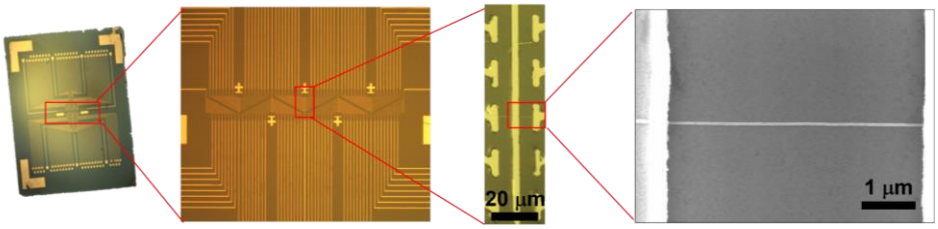

Fig. S2. Optical and SEM images of a high-density SiNW FET array. We designed a pattern with 96-pairs electrodes to make transistor arrays.

II. Electrical characterization

The FET properties of these functionalized SiNW FETs were characterized at ambient room temperature by using an Agilent 4155C semiconductor analyzer and a Karl Süss (PM5) manual probe station. Heavily doped Si substrates were used as the global back gate. Before and after the photolithographical patterning and e-beam thermal deposition processes, the transfer curves of SiNW FETs did not show significant changes as shown in Fig. S3A. These devices were turned on at nearby 0 V under liquid gate modulation with the sensitivity of ~800 nA/V and the subthreshold swing of ~100 mV/dec (Figs. S3B and S3C).

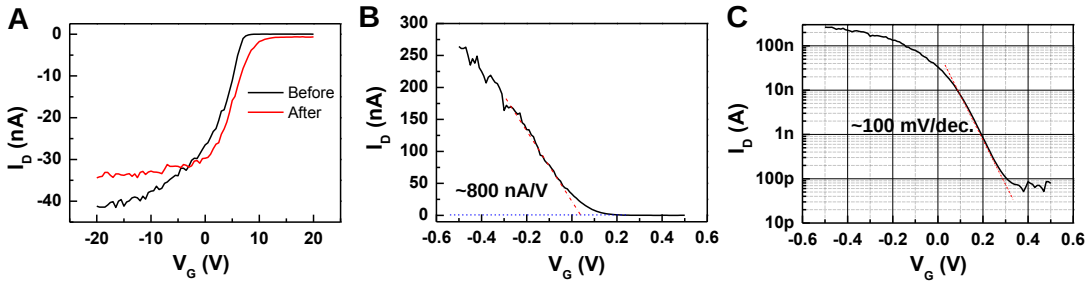

Fig. S3. (A) Transfer curves of a SiNW FET before and after the photolithographical patterning and e-beam thermal deposition processes. (B) Sensitivity of a SiNW FET under a liquid gate. (B) Subthreshold swing shown at semi-logarithmic coordinate. V_D = 0.1 V.

III. More details of XPS characterizations

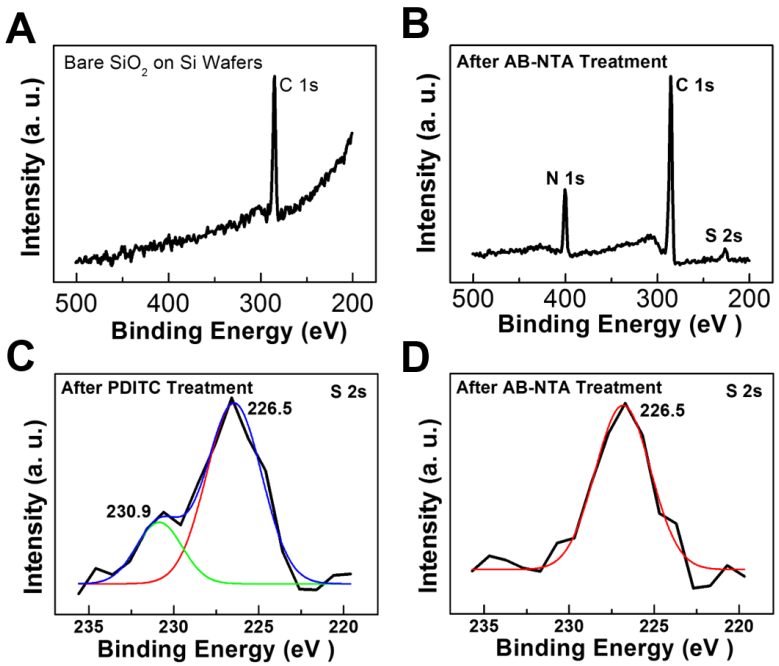

Fig. S4. XPS spectrum of the bare SiNWs (A) and after further AB-NTA treatment (B). High-resolution S 2s core level spectra of silicon wafers after PDITC treatment (C) and after AB-NTA treatment (D).

Table 1. Comparisons of calculated and experimental elemental ratio in each step

Elemental ratio	N-APTMS	PDITC	AB-NTA
C/N (Calculated)	2.50	3.25	3.83
C/N (Experimental)	2.75	2.98	3.15
C/S (Calculated)	/	6.50	11.50
C/S (Experimental)	/	6.72	11.69
N/S (Calculated)	/	2.00	3.00
N/S (Experimental)	/	2.26	3.51

IV. F_1-ATPase detection

To confirm the selective adsorption of motor proteins on the SiNW surface, a drop of tris-buffer solution of F_1-ATPase (0.5 μM) was placed on the functionalized devices at 4 ^oC for 2 h. After thoroughly rinsing the devices by the pure tris-buffer solution and deionized water, we characterized the protein distribution by AFM. As shown in Fig. S5A and S5B, apparently the substrate is clear and the protein particles are distributed only on the SiNW surface. Control experiments where we used the devices that were functionalized by N-APTMS after SiNW transfer and device fabrication on the whole surface (including the substrate) show that the protein particles are distributed uniformly on the whole surface of silicon wafers (Fig. S5C and S5D). These results consistently prove the highly selective chemical modification of our strategy, thus ensuring the detection success in solutions with ultralow concentration.

By using a home-made PDMS microchannel to introduce the tris-buffer solution of F_1-ATPase (~1.84 nM) (Fig. S6), we carried out real-time electrical measurements at the constant temperature (25.0 ^oC), which was controlled by a temperature control system (Instec mK2000). Before protein delivery, a pure tris-buffer solution (pH = 8.0) was introduced to stablize the device for at least 100 s. Fig. S7 shows that the long-time treatment fo the pure tris-buffer solution did not lead to the obvious current changes. Real-time measurement data were collected by using a Lock-in Amplifier (HF2LI Lock-in Amplifier, Zurich Instruments) with a preamplifier (DL1211, DL Instruments) and with a sampling rate of 3600 Sa/s. We also characterized the electrical properties of the same SiNW FET before and after multiple protein adsorption. As shown in Fig.

S8, we observed the obvious change in their transfer curves before and after protein adsorption. This is in good consistence with real-time measurements of individual protein adsorption as demonstrated in Fig. 4.

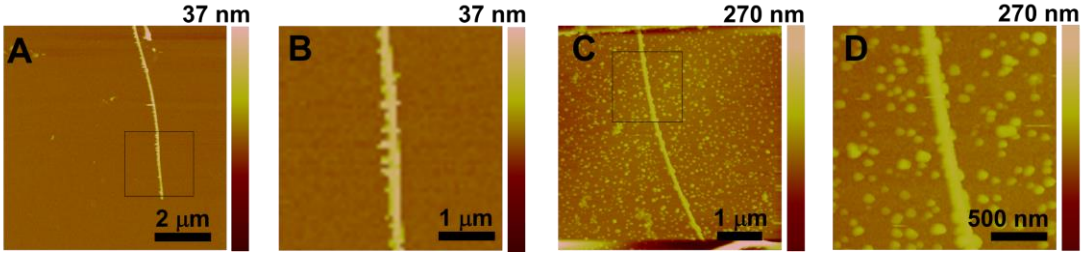

Fig. S5. (A) AFM image of protein distributions by using the highly selective chemical modification procedure. (B) Enlarged AFM image in Fig. S5A. (C) AFM image of protein distributions of control experiments. (D) Enlarged AFM image in Fig. S5C.

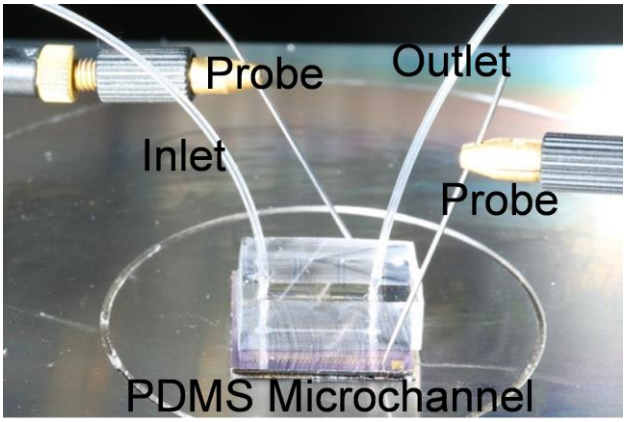

Fig. S6. Optical image of a home-made PDMS microchannel used for real-time measurements.

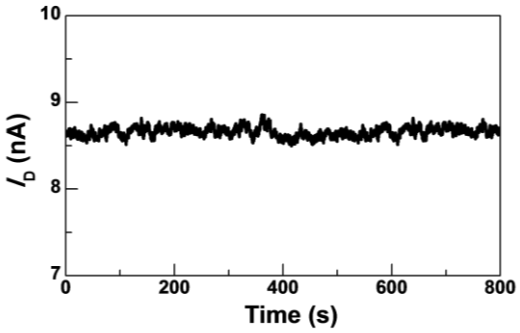

Fig. S7. Real-time current detection of a device treated by a pure tris-buffer solution.

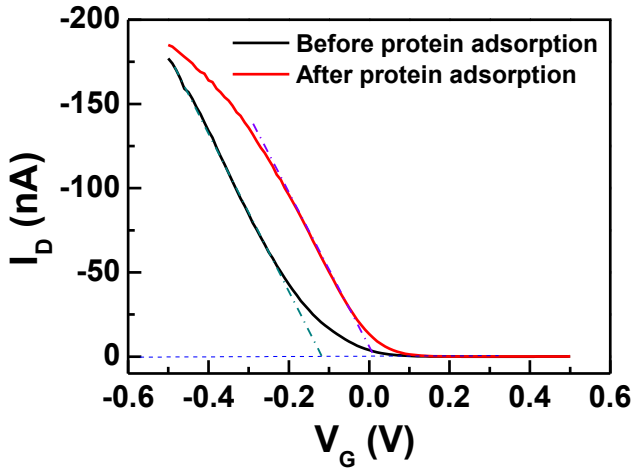

Fig. S8. Transfer curves of a SiNW FET before and after multiple proteins adsorption. V_D = 0.1 V.

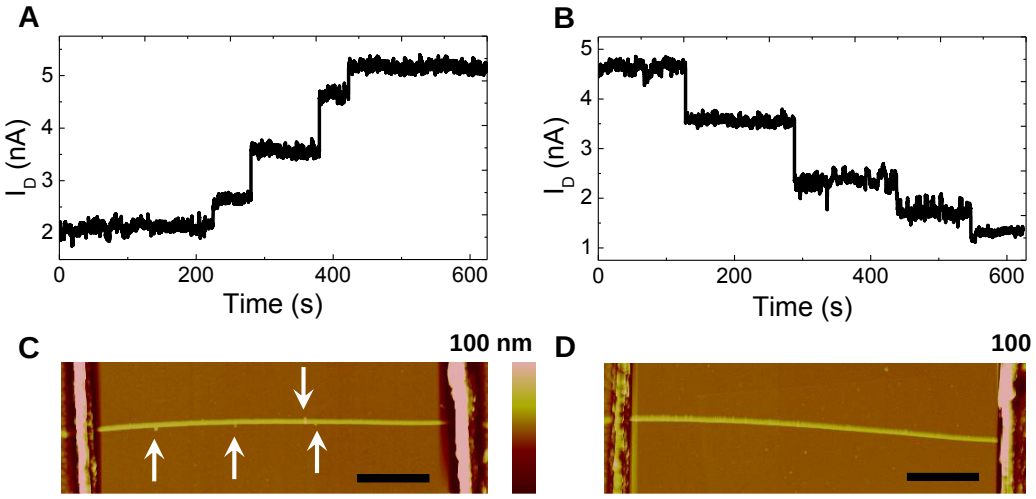

Fig. S9. Step increase/decrease signals at another device. (A-B) Real-time recordings of the absorption/desorption processes of F_1-ATPases, showing the gradual changes in I_D with four steps. $V_D = 0.1$ V and $V_G = 0$ V. (C-D) Corresponding AFM images after proteins delivery (C) and after further EDTA treatment (D). The scale bar is 1 um.

V. References

1. F. Patolsky, G. Zheng and C. M. Lieber, *Nat. Protoc.*, 2006, **1**, 1711.

2. F. X. Shen, J. D. Wang, Z. Q. Xu, Y. Wu, Q. Chen, X. G. Li, X. Jie, L. D. Li, M. S. Yao, X. F. Guo and T. Zhu, *Nano Lett.*, 2012, **12**, 3722.

3. J. D. Wang, Z. X. Wang, Q. C. Li, L. Gan, X. J. Xu, L. D. Li and X. F. Guo, *Angew. Chem. Int. Ed.* 2013, **52**, 3369.

4. J. Wang, F. Shen, Z. Wang, G. He, J. Qin, N. Cheng, M. Yao, L. Li and X. Guo, *Angew. Chem. Int. Ed.*, 2014, **53**, 5038.

5. J. Yao, H. Yan and C. M. Lieber, *Nat. Nanotechnol.*, 2013, **8**, 329.