Supporting information

A dynamic reversible phenylboronic acid sensor for real-time determination protein-carbohydrate interactions on living cancer cell

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1.Materials and Instruments

Dopamine hydrochloride, concanavalin A (Con A), D-galactose, tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma. Wheat germ agglutinin (WGA), ulex europaeus agglutinin I (UEA-I), soybean agglutinin (SBA), and peanut agglutinin (PNA), were purchased from Vector Labs. N-acetylglucosamine (GlcNAc), 2,2'-(ethylenedioxy)bis(ethylamine), D-mannose, and L-fucose were obtained from Sarn Chemical Technology (Shanghai, China). 4-carboxyphenylboronic acid (CPBA) was obtained from Aladdin amd the fluorescent stain Hoechst 33258 was obtained from Solaibio. Human acute T lymphocytic leukemia cells (Jurkat) and Human chronic myelogenous leukemia cells (K562) were generous gifts from Prof. Bin Gao (Institute of Microbiology, Chinese Academy of Sciences, Beijing, China).

Bruker 500 MHz Spectrometer (BrukerDaltonics Inc., Billerica, MA, USA) recorded ^{1}H NMR spectra, using residual signals from D_2O (^{1}H: δ 4.79 ppm) or $CDCl_3$ (^{1}H: δ 7.26 ppm) as internal standards. Gold sensor chips with 10 MHz AT-cut quartz crystals were obtained from Attana AB. Biosensor experiments were undertaken using an Attana Cell A200 QCM instrument (Attana AB, Stockholm, Sweden) at 20 ℃ with PBS at pH 7.4 as running buffer.

1.1 Polydopamine and phenylboronic acid derivative (PBA) coating chip

The gold sensor chips were washed with 1% NaClO, piranha solution (98% H_2SO_4:30% H_2O_2, 3:1, v/v), and ultrapure water, respectively. The dry gold chip was incubated with 2 mg/mL of dopamine solution (in 10 mM Tris-HCl buffer at pH 8.5) for 1.5 h at room temperature to form polydopamine by self-agglomeration and adsorbed on the surface of the chips. The polydopamine-coated chips were washed by ultrapure water and dried under nitrogen (N_2). Next, 50 μL of compound **3** (phenylboronic acid derivative, PBA) was diluted to 50 mM (in 10 mM Tris-HCl buffer

at pH 8.5) and added dropwise to the polydopamine-coated chip surface, incubated for 4 h at room temperature. Then PBA was fixed on the polydopamine-coated chip by Michael addition and Schiff base reactions. At last, the PBA functionalized chip (PBA-chip) were then washed three times with PBS at pH 7.4 and dried with N_2.

1.2 Surface characterization

The thickness of the polydopamine-coated chip was measured by a PZ 2000 ellipsometer (Horiba, France). The surface morphology and roughness of the sensor surface were characterized using an atomic force microscope (AFM, Multimode-8, Bruker Corporation, USA) in ScanAsyst mode. And the chips were determined by scanning electron microscopy X-ray energy spectrometry (SEM-EDS) (Hitachi, Japan).

1.3 Preparation of living suspension cancer cell sensor chips

The suspension cells Jurkat and K562 were diluted with PBS to 1.4×10^6 cells/mL, respectively. The cells were incubated on the PBA-coated chips at 0.5 ml per chip at room temperature for 1 h to prepare living suspension cell sensor chips. The suspension cells on the sensor were stained by Hoechst 33258 and the active state of the cells and coverage on the sensor surface were observed by a fluorescent microscope (Olympus BX53).

1.4 QCM detection

The prepared suspension-cell biosensor was docked into the Attana Cell A200 QCM instrument, and then stabilized with 10 mM pH 7.4 PBS as running buffer at a flow rate of 20 μL/min. The instrument was stable and the detection was initiated when the frequency baseline drift was within 0.5 Hz/min. A series of lectins dissolved in running buffer were injected into the QCM instrument at the concentration of 50 μg/mL to evaluate the interactions between lectins and living suspension cell surface glycans. After lectins injection, PBS running buffer was flowing onto the sensor surface for 300 s. Then the corresponding monosaccharide solution was injected at the concentration of 300 mM to regenerate the cell sensor. A competitive experiment was conducted using D-mannose as the competitor, which is a known ligand bound to Con A. The 50 μg/mL Con A solution was mixed with a series concentration (0-495 mM) of D-

mannose at a volume ratio of 1:1, and then the mixture was injected into QCM instrument and measured. For the kinetic study, the lectins were diluted with PBA to 25, 50, 75 and 100 μg/mL, respectively and then introduced onto the suspension-cell sensor surface three times with intermediate regeneration steps.

1.5 Data process

The changes of resonance frequency of the suspension-cell sensors were measured and recorded in real-time by Attana AB and its Attester software. The experimental data of the competitive experiment and the kinetic study were fitted with the evaluation software (Attana AB) and ClamXP.

2. Cell culture

Jurkat and K562 cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) FBS, and 1% penicillin-streptomycin at 37 ℃ with 5% CO_2 and 95% humidity. To prepare cell suspensions, Jurkat or K562 cells in logarithmic growth phase were removed from the culture medium by centrifugation. The cell density was diluted to 1.4×10^6 cells/mL after three washes with PBS. The modified QCM chips were placed in a 24-well plate, and 0.5 mL of the above cell suspension (containing 7×10^5 cells) was added and incubated in an incubator for 1 h to fix them on the chip surface [1]. After incubation, the unfixed suspension cells were removed by washing with PBS, and the cells fixed on the surface of the chip were stained to detect the cell coverage. Finally, the chips were mounted on the cell sensing chip holder and set aside for use [2].

3. Fitting equation

The curve fitting was performed by Graphpad Prism 5 software using the following equation:

$$\Delta f = \Delta f_{min} + \left(\frac{\Delta f_{max} - \Delta f_{min}}{1 + 10^{(logC - logIC50)}}\right) \qquad \text{Equation (2-1)}$$

Where: Δf mean response frequency Hz; Δf_{max} intended to be the maximum Hz of the response frequency; Δf_{min} mean response frequency nadir Hz; C representative inhibitor concentration M; IC_{50} mean half inhibitory concentration M.

4. Characterizations of compound

4.1 Compound 1

15 mL of dry tetrahydrofuran (Dry THF) and 1.9 g (13 mmol) of 2,2'-(ethylenedioxy)bis(ethylamine) were added to a 50 mL round-bottom flask sequentially. 0.85 g (3.9 mmol) di-tert-butyl dicarbonate (BoC_2O) was dissolved in 10 mL of Dry THF and slowly added dropwise to the reaction solution in a round bottom flask over 30 min at 0 °C. The reaction was carried out at room temperature for 12 h and detected by thin-layer chromatography (TLC), and the reaction was terminated after the complete reaction of the raw materials. After removing the organic solvent by distillation under reduced pressure, 50 mL of ethyl acetate (EA) was added, followed by 30 mL of saturated sodium chloride solution washed three times (3×30 mL), and the organic phase was collected. The organic phase was dried by adding 10 g of anhydrous sodium sulfate (Na_2SO_4) and removed by filtration at the end of the treatment. The organic solvent EA was removed by distillation under reduced pressure and analyzed by column chromatography to give compound **1** as a light yellow transparent oil with a mass of 0.86 g and a yield of 89%. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) δ/ppm = 5.30 (s, 1H), 3.72 (s, 4H), 3.66 (m, 4H), 3.57 (q, J = 5.0 Hz, 2H), 2.34 (t, J = 5.0 Hz, 2H), 1.44 (s, 9H) [3].

4.2 Compound 2

1 g (4 mmol) of compound **1**, 0.8 g (4.8 mmol) of 4-carboxyphenylboronic acid, 97 mg (0.8 mmol) of 4-dimethylaminopyridine (DMAP) and 0.99 g (4.8 mmol) of

dicyclohexylcarbodiimide (DCC) were added sequentially to a 50 mL round bottom flask, charged with nitrogen (N_2) and protected by N_2. The reaction was terminated by adding 20 mL of dry dichloromethane (Dry DCM) at room temperature for 24 h. The reaction of compound **1** was detected as complete by TLC. Add 30 mL of DCM, followed by the same 30 mL of saturated sodium chloride solution washed three times (3×30 mL), collect the organic phase, add 5 g of Na_2SO_4 to dry the organic phase and remove it by filtration at the end of the treatment, distill under reduced pressure to remove the organic solvent DCM, and carry out column chromatography separation to give compound **2** with a mass of 0.74 g and a yield of 46.7%. ^{1}H NMR (500 MHz, $CDCl_3$) δ/ppm= 8.56-7.55 (m, 4H), 3.73 (s, 2H), 3.72-3.57 (m, 8H), 3.52 (s, 2H), 3.26 (s, 2H), 1.42 (s, 9H) [3].

4.3 Compound 3

0.74 g (1.9 mmol) of compound **2** was added to 5 mL of Dry DCM in a 25 mL round bottom flask charged with N_2. The reaction was terminated by stirring at room temperature for 5 h. The organic solvent DCM was removed under reduced pressure and 20 mL of methanol (MeOH) was added three times (3×20 mL) to obtain yellow oil compounds with a mass of 0.72 g. All the yellow oil was dissolved in 25 mL of MeOH and slowly add saturated sodium carbonate solution dropwise until the pH is neutral. The filtrate was filtered, removed, washed three times with 10 mL of MeOH, and the organic solvent was removed under reduced pressure to give a white solid as compound **3**, with a mass of 0.55 g in 99% yield. ^{1}H NMR (500 MHz, D_2O) *δ*/ppm= 7.83 (s, 4H), 4.00 - 3.80 (m, 6H), 3.74 (d, J = 16.1 Hz, 4H), 3.64 (s, 1H), 3.36 (s, 1H), 2.96 (t, J = 5.1 Hz, 2H), 2.84 (s, 1H). Compound **3** is a known compound and the known NMR data are ^{1}H NMR (400 MHz, DMSO-d_6) *δ*/ppm= 8.54 (t, J = 5.2 Hz, 1H), 7.85 (d, J =

8.2 Hz, 2H), 7.79 (d,J = 8.2 Hz, 2H), 3.66-3.50 (m, 8H), 3.43 (q, J = 5.7 Hz, 2H), 2.95 (m, 2H) [3].

5. Figure S1-S10

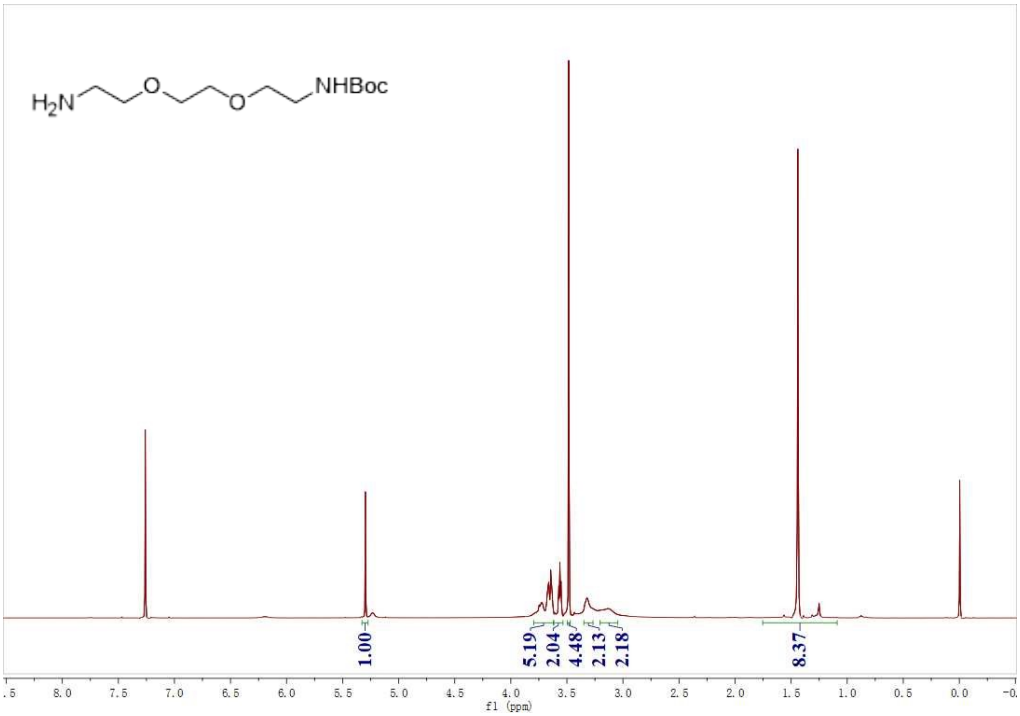

Figure S1. The ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) of compound **1**.

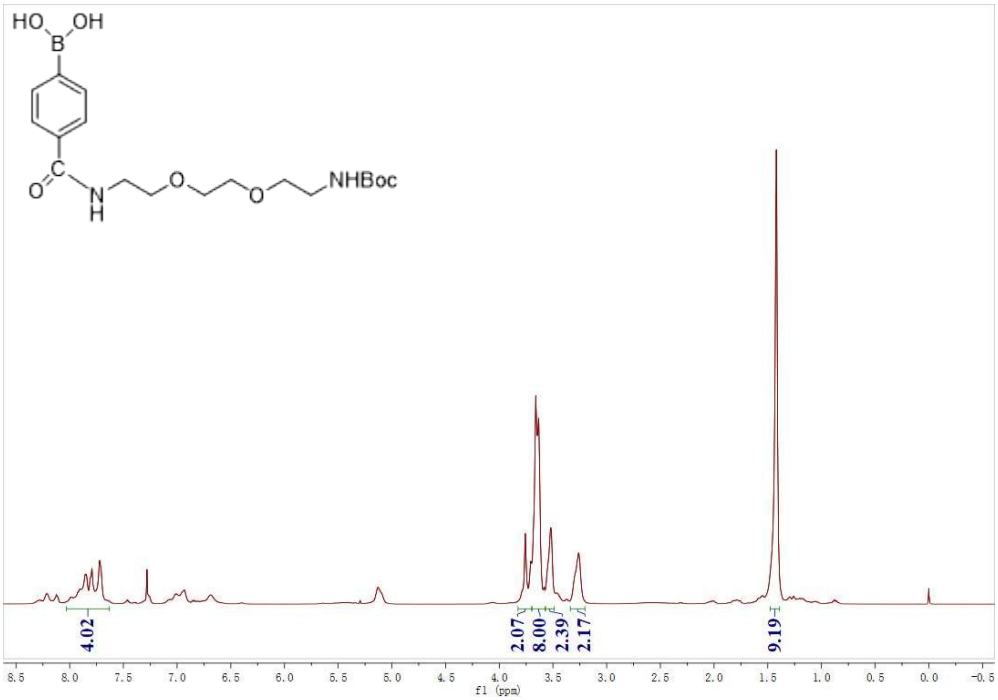

Figure S2. The ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) of compound **2**.

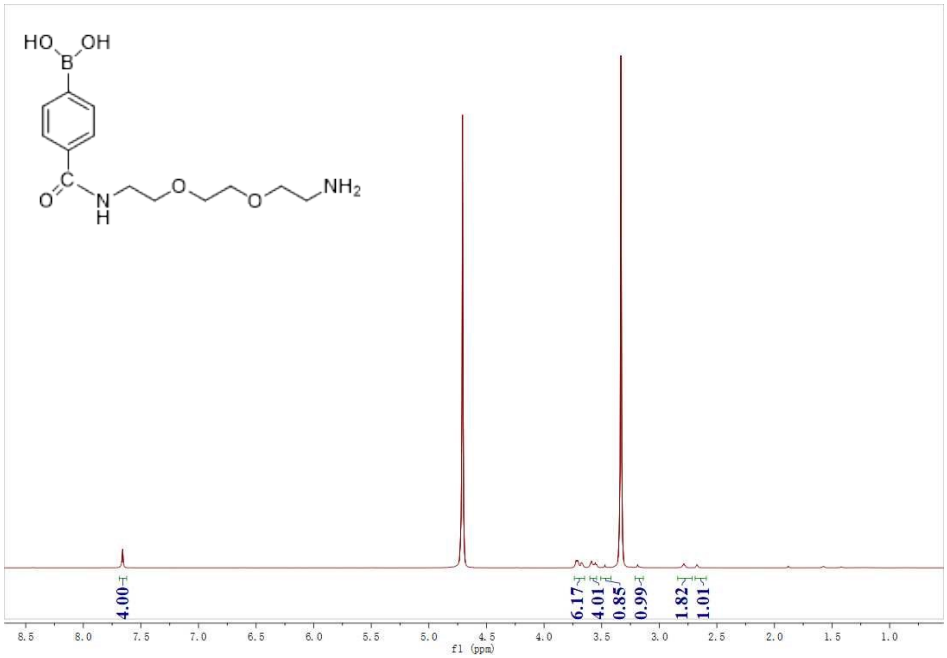

Figure S3. The ^{1}H-NMR spectrum (500 MHz, D_2O) of compound **3**.

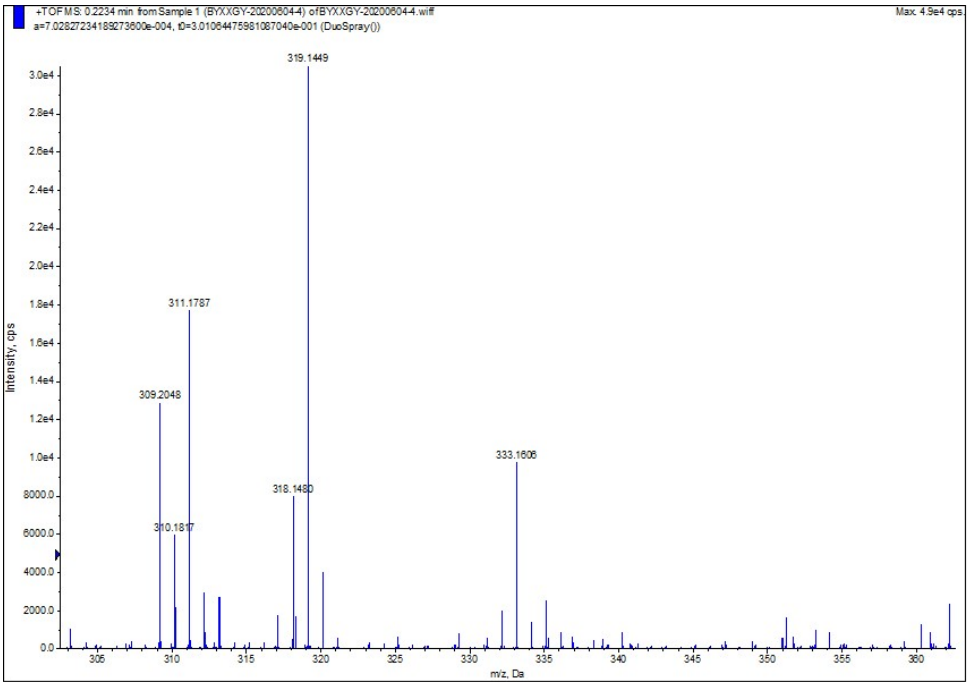

Figure S4. The HRMS spectrum of compound **3**.

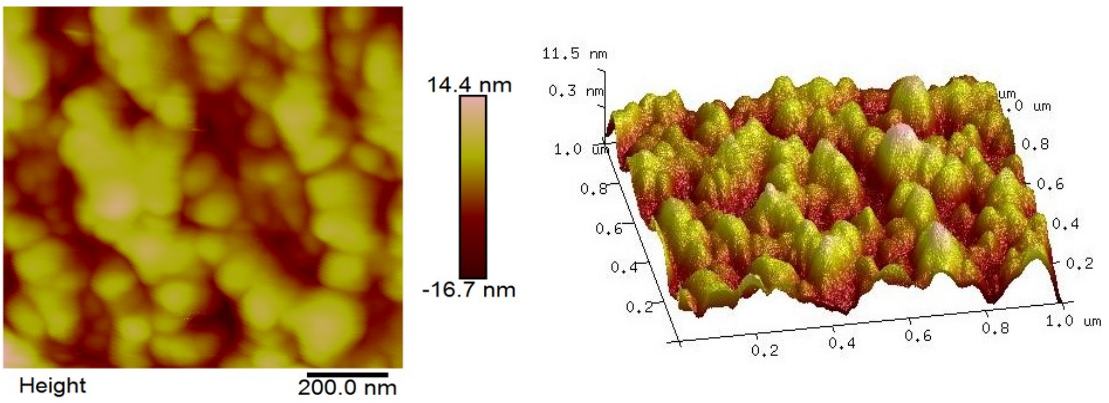

Figure S5 AFM measurement of gold chip.

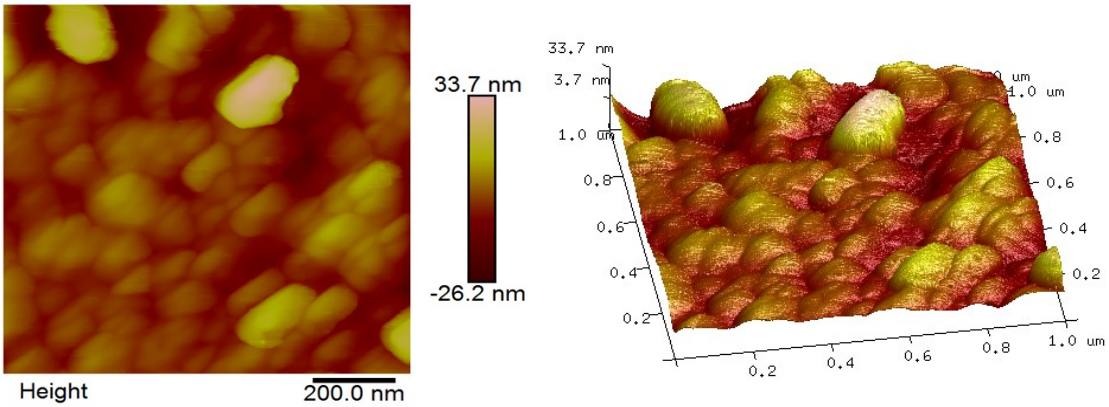

Figure S6 AFM measurement of polydopamine coating chip.

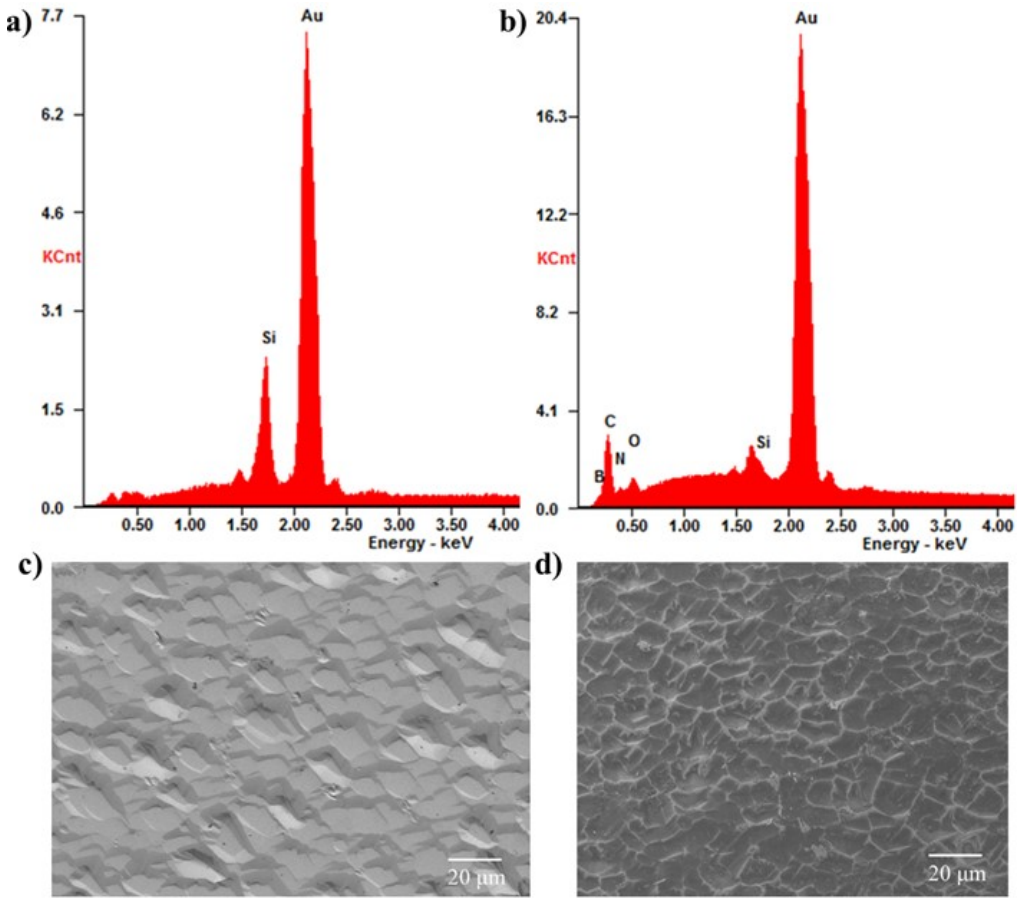

Figure S7. The SEM-EDS spectra of (a) gold chip surface and (b) PBA-chip surface, The SEM image of (c) gold chip surface and (d) PBA-chip surface.

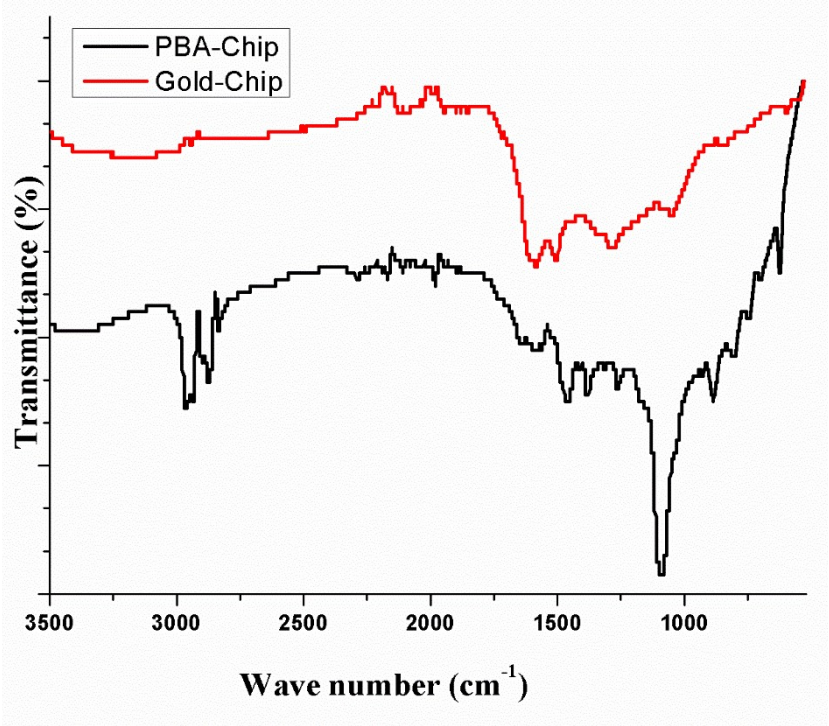

Figure S8. The FT-IR spectrum of PBA functionalized chip (black) and gold chip (red).

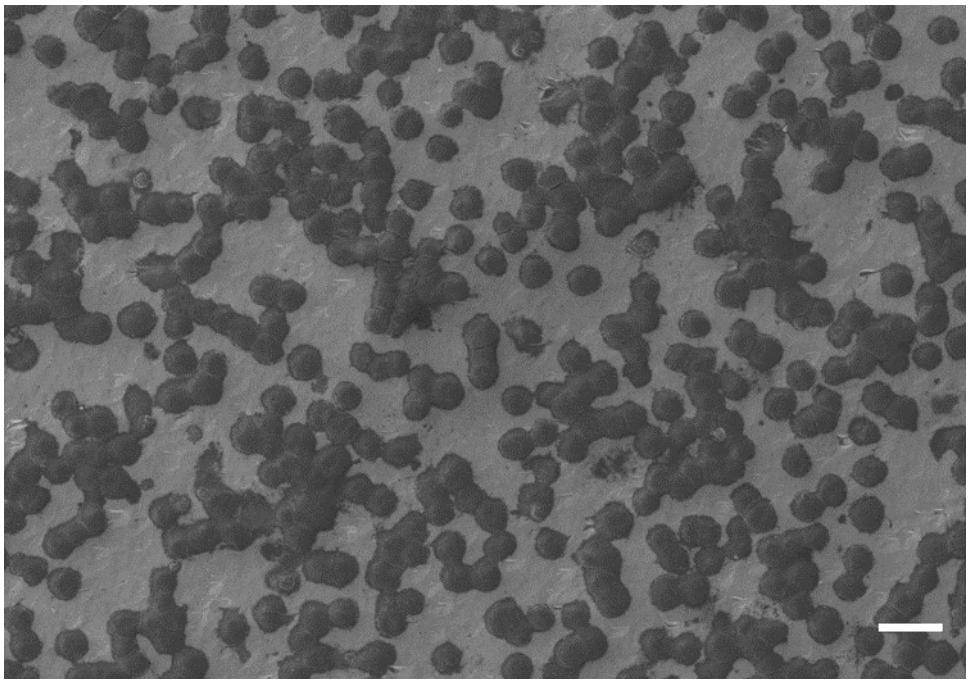

Figure S9. The SEM image of the PBA-based suspension-cell sensor surface, on which the Jurkat cells were captured by boronic acid groups and fixed on the chip. The scale bars stand for 40 μm.

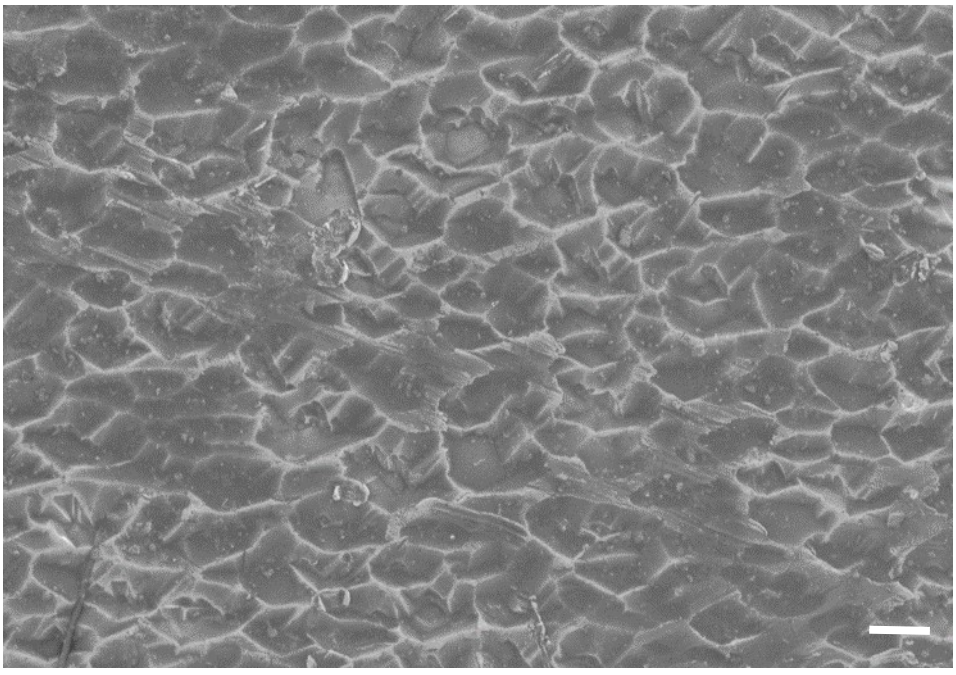

Figure S10. The SEM image of the regeneration of PBA-chip surface. The scale bars stand for 10 μm.

References

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[2] X.M. Li, Q.Q. Song, Y.X. Pei, H. Dong, T. Aastrup and Z.C. Pei, Direct attachment of suspension cells to PDA surface and its application in suspension-cell QCM biosensor, Sensor. Actuat. B-Chem. 326 (2021) 128823.

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