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Supplementary information

Detection of cell membrane proteins using ion sensitive field effect transistors combined with chemical signal amplification

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Cell culture

Human breast cancer cell lines (BT474, MCF10A, and MDA MB 231) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). MCF10A cells were cultured on a cell culture dish in MEGM™ Mammary Epithelial Cell Growth Medium (Lonza, Walkersville, MD, USA). Other cells were cultured in Dulbecco's RPMI 1X medium with 10% fetal bovine albumin (FBS), 1% penicillin, and Glutamax™ 1X. The cell culture environment was 37°C in 5% CO_2.

Immunofluorescence for imaging HER2 expressed on breast cancer cell lines

The BT474, MCF10A, and MM231 breast cancer cell lines were trypsinized and seeded to a glass bottom dish for confocal microscopy observation. The solution for antibody dilution was 1 w/v% bovine serum albumin (BSA) in phosphate buffer saline (PBS) and the blocking solution was 10 v/v % normal goat serum (Vector Laboratory, Burlingame, CA, USA), 0.2 v/v % Tween 20, 0.7 v/v % glycerol, and 2% BSA in PBS. After the cells were incubated at 37°C and 5% CO_2 overnight, 4 v/v% paraformaldehyde in PBS was applied to fix the cells for 20 minutes, and then the cells were washed with PBS three times. Blocking solution was then added and incubated for 1 hour, and then a 1:200 diluted solution of a rabbit anti-HER2 polyclonal antibody (Sigma-Aldrich Co. LLC, Burlington, MA, USA) was applied and incubated for 1 hour. A 1:400 diluted solution of anti-rabbit labeled FITC was then applied and incubated for 1 hour after a PBS washing step. 4,6-diamidino-2-phenylindole (DAPI; 1:1000 dilution in PBS; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) was introduced to the cells and incubated for 10 minutes. After the washing step, the HER2 expression was observed with a Nikon Eclipse Ti-E confocal microscope using a LU-N series laser unit (Nikon, Tokyo, Japan).

Cell seeding on the ISFET gate surface

An open gate n-channel depletion type ISFET with 40-nm thick Ta_2O_5, 140-nm thick Si_3N_4, and 125-nm thick SiO_2 layers as a gate insulator (Isfetcom Co. Ltd.,

Saitama, Japan) was used. The glass chamber (6 mm inner diameter) was attached to the gate surface using thermosetting epoxy resin (120°C, 2 hours). To decontaminate the gate surface, the sensor surface was cleaned using 200 µl of the 60 µl NH_4OH (10 v/v %) and 140 µl H_2O_2 (30 v/v %) mixture, and rinsed with deionized water. The ISFET gate was then pretreated with 100 µl of poly-L-lysine aqueous solution (0.01 w/v%) for 10 minutes. Deionized water was used for rinsing and then the sensor was incubated for 2 hours at 60°C. The cells (10^5 cells/ml) were trypsinized and seeded on the poly-L-lysine pretreated gate. The sensor was then incubated at 37°C in 5% CO_2 overnight. The cell seeded ISFET was observed with microscopy and used for the following ISFET experiments.

Detection of HER2 via the urea–urease reaction using ISFET

A LabJack U6-Pro (LabJack Corp., Lakewood, CO, USA) circuit controller was used to measure the surface potential of the gate against an Ag/AgCl reference electrode with salt bridge. The drain–source current was 500 µA and the drain source voltage was 530 mV. The sampling period was 1 second. Each of the standard solutions (pH 4, 7 and 9) were applied to the ISFET chamber for 5 minutes to measure the ISFET potential response.

The rabbit anti-HER2 polyclonal antibody (Merck, Darmstadt, Germany) was applied to the BT474 cells captured on the ISFET gate. After gentle rinsing of the ISFET gate with PBS buffer three times to remove unbound antibody, the anti-rabbit IgG urease conjugate (Merck, Darmstadt, Germany) (1:1,600 diluted) secondary antibody was added and incubated for 90 minutes. After washing the gate surface with the PBS, the modification process on the ISFET gate was complete and the device was ready to detect HER2 expression. All of the data were analyzed in comparison with those of bare ISFET (no enzymatic reaction).

Fig. S1 The ISFET used for detection of cell membrane proteins. a) pH sensing area of the ISFET and b) the measurement circuit.

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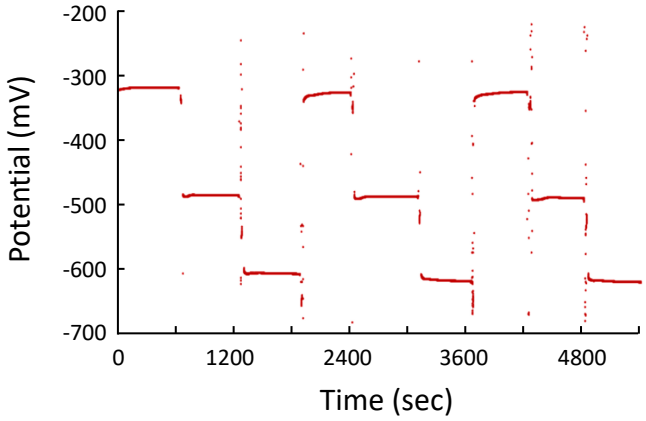

Fig. S2 Typical pH sensitivity evaluation data of the ISFET in pH 4, 7, and 9 standard buffers.

To make a pH sensitivity calibration curve, the output potential of ISFET was monitored in real-time at pH 4, 7, and 9. The ISFET response data were analyzed using the Nernst equation and the sensitivity was 57.2 mV/pH.

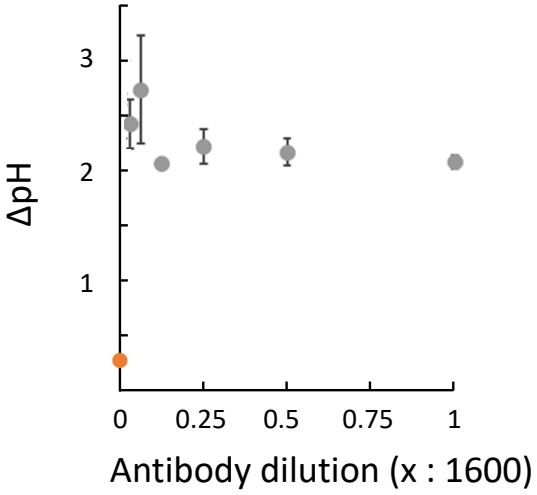

Fig. S3 Activity confirmation of urease conjugated secondary antibody. Relationship between the pH change included by the urea–urease reaction and the amount of urease conjugated secondary antibody.

Fig. S3 shows the relationship between the different amounts of anti-rabbit IgG conjugated urease and pH change. The pH changes generated by a urea–urease reaction was monitored with ISFETs with different amounts of anti-rabbit IgG conjugated urease. The series of anti-rabbit IgG conjugated urease was diluted at a ratio of 0.03125:1600 to 1:1600 with 0.05 mM PBS. Here, the lower buffer concentration is suitable for bigger pH changes, which would provide a minimum buffer capability and provide electrical conductivity in the measurement system. One hundred microliters of the diluted antibody solution was applied to the ISFET chamber and the potential response of the ISFET was measured. After the ISFET potential response became stable, a 15 mM urea solution was introduced to the ISFET chamber. After introducing a urea solution, a pH shift of approximately 0.2 was observed even without the labeled urease. This pH change was induced by the pH difference between the urea solution and 0.05 mM PBS. In the case with the labeled urease, a pH shift around 2–2.5 was observed because of the enzymatic reaction. As shown in Equation 2, ammonium ion production leads to a higher pH in the solution. Therefore, the catalytic activity of the labeled urease was confirmed in the solution phase. Although the pH shift was maximized at a dilution ratio of

0.06:1600, there was no significant difference in the pH shift at the dilution ratios considered. Because the expression level of HER2 per cell is unknown in this study, when applying this antibody-modified urease to HER2 detection, it is preferable to have a large amount of urease to obtain a large signal change. Therefore, we decided to use the maximum dilution ratio (1:1600) in ISFET experiments.