Supplementary Information

Monitoring the heterogeneity in single cell responses to drugs using electrochemical impedance and electrochemical noise

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Experimental Methods

Materials

Antibody Anti-EpCAM IgM [4G10] and Asante Potassium Green-2 (APG-2) AM were purchased from Abcam (Cambridge, MA, USA). Hoechst 33342 was purchased from Invitrogen. Doxorubicin hydrochloride was purchased from Sapphire Bioscience.

Silicon substrates were prime grade, single-side polished, 100-oriented (<100> ± 0.9°) poorly doped p-type silicon (10-20 Ω cm, 500 ± 25 μm thick). As shown in Supplementary Figure 1, the surface was firstly passivated by an alkyne-terminated organic monolayer on hydrogen terminated silicon *via* thermal hydrosilylation[22]. This is a well-established technique to prevent oxidation of the silicon in aqueous during electrochemistry and perform light activated electrochemistry[3]. Followed antibodies that can quickly bind to surface antigens on target cells were attached on silicon through a custom synthesized linker molecule, which will allow efficient cell attachment to silicon electrode and is vital to perform cell-surface impedance[24].

Cell culture

MCF-7 cell line was purchased from CellBank Australia (Westmead, NSW, Australia) and tested with MycoAlert™ Mycoplasma Detection Kit (Lonza Group) and found to be mycoplasma negative. The doxorubicin resistant MCF-7 cell line was adapted to continuous exposure to 100 nM doxorubicin. Parental MCF-7 and doxorubicin resistant MCF-7-VP cell lines were propagated in the Dulbecco's Modified Essential Media (DMEM, Life Technologies) medium supplemented with 10% heat inactivated fetal bovine serum (FBS, Life Technologies) and 1% antibiotic–antimycotic (Sigma). The cells were grown in 5% CO2 at 37°C. Cell concentration and viability analysis were performed using a Trypan Blue dye exclusion assay (mix 10 μL cell suspension with 10 μL Trypan Blue (Sigma), and pipette 10 μL of the mixture into the two sides of haemocytometer, count and average them). For the determination of the viability of cells on silicon surfaces, 0.5 % Trypsin were used to detached the cells.

A silicon sample was placed in a home-made PTFE chamber (after sterilization) sealed with O-ring. The chamber was rinsed with warm cell growth medium twice and reloaded with medium at a density of 1×10^5 cells mL^{-1}. The chamber was covered with a lid and then placed in the 37 °C incubator for 1 h. The silicon surface with cell layer in the electrochemical chamber was rinsed with warm cell media twice before further cell impedance experiments.

Cell imaging and impedance measurement

The bright field and fluorescence images were collected using OLYMPUS BX53 upright microscope and FV1200 confocal microscope with an Olympus water immersion objective (40x, NA 0.80). Hoechst 33342 (1:1000) was used to label DNA, and excited by ultraviolet light at 350 nm. Cell images with doxorubicin showed red fluorescence excited at 480 nm and detected at 590 nm. For in *vitro* characterization of K^+ concentration of the MCF-7 cells, 1 μM APG-2 AM was used to stain the cells for 30 min and low-light level fluorescence imaging was excited at 520 nm and detected at 550 nm. ImageJ was used for cell area measurement, and the detailed tutorial is: go to 'Image'→ 'Adjust' → 'Threshold'; then go to 'Analyze'; then go to Analyze → 'Tools' → 'ROI manager' → select cells of interest, then click 'Measure'. 'Results' window pops up with the areas of cells measured. In 'Results' window, go to 'Results' → 'Options' and I/O option box pops up. In the I/O option box, type '.xlsx' in 'file extension for tables'. Select all four 'Results Table Options' and click 'OK'. Go to 'File' → 'Save as', the Excel file could be saved.

By setting the system to a three-electrode system and coupling the microscope with a potentiostat (SP-200, BioLogic Science Instrument), single-cell impedance data were acquired when localizing the laser beam to one specific cell. By sweeping a AC potential with different frequencies (400 Hz, 4000 Hz, 40K Hz) and simultaneously recording the resulting impedance value, one can obtain impedance *vs.* potential plots. By comparing the impedance difference between impedance *vs.* potential plots from a cell region and cell free region, we selected a typical AC potential and frequency (E = 0.1 V, f = 400 Hz) that a ~0.8-1.5 Ω difference is easily obtained, and used them for single-cell impedance recording through the work.

Fast Fourier transforms and Statistical analysis

Fast Fourier transforms (FFT) were applied to find the frequency components of signals buried in impedance noise, and the FFT analysis was performed in MATLAB. In FFT amplitude spectrum of the impedance data, the frequency with highest amplitude is the domain frequency, so the corresponding amplitude at this domain frequency is compared through the work. Unless otherwise specified, in box plots the boxes capture the interquartile range, the center line denotes the median.

Supplementary Figures

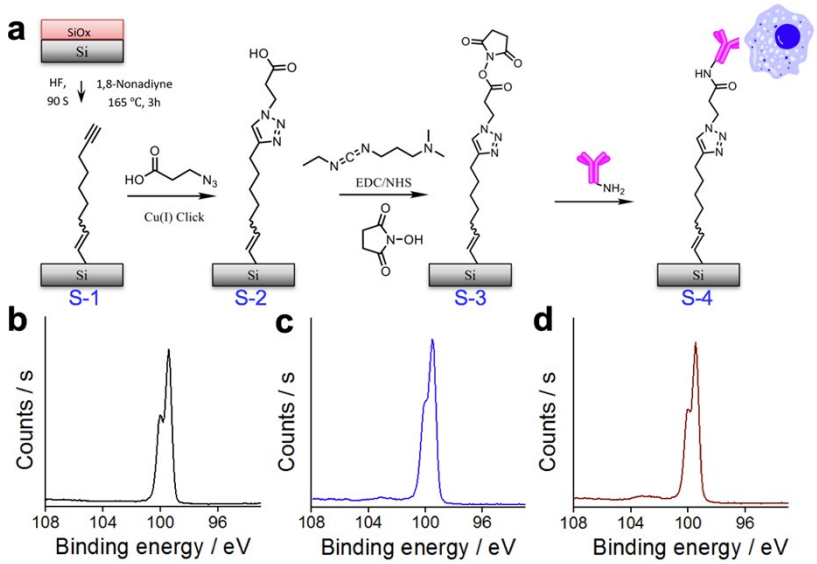

Fig. S1. (**a**) Customized surface chemistry for light addressable single-cell impedance. A native silicon surface is etched with 2.5 HF for 90s, thermal hydrosilylation of 1,8-nonadiyne is used to chemically passivate the hydrogen terminated silicon. 3-Azidopropionic acid is grafted on the acetylenylated silicon *via* CuAAC click reactions to generate a linker that can react with the primary amine group on antibody EpCAM *via* carbodiimide crosslinking reaction in the presence of EDC/NHS. The receptor molecule overexpressed in the MCF-7 cells then binds to EpCAM antigens on the silicon surface. The obtained acetylenylated silicon, carboxyl terminated silicon, anti-EpCAM attached silicon, and MCF-7 attached silicon was termed as S-1, S-2, S-3 and S-4, respectively. The obtained silicon surface S-1, 2, and 3 could be stored under argon atmosphere for weeks prior to use. High-resolution XPS spectrum of Si 2p regions for the silicon electrode used for cell impedance sensing (S-4) prior to incubated in cell-culture medium (**b**), after 3 h incubation in cell-culture medium (**c**), and after 3 h incubation and 2 h impedance measurement in cell-culture medium (**d**).

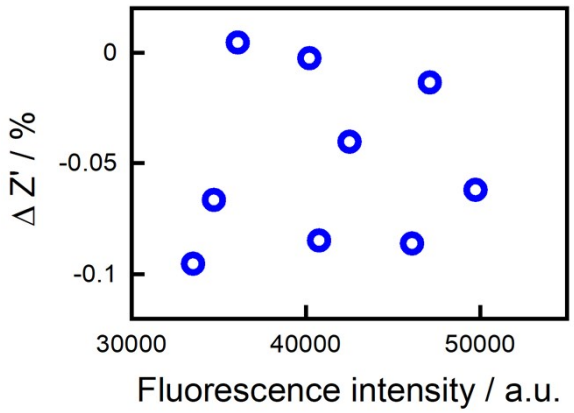

Fig. S2. Plot of the variation in single cell impedance versus the fluorescence intensity, which serves as a measure of the amount of doxorubicin taken up by the cells for the same 9 cells as in Fig. 2e. Cells captured on the silicon surface and underwent treatment with 1 µM doxorubicin for prior to the measurement.

Supplementary Table 1. Cell viability for cells under different treatment conditions

Treatment group	Cell viability (%)
Cells before incubation on silicon	96.7 ± 3.2
Cells incubated on silicon after 1h impedance performance	95.1 ± 7.3
Cells incubated on silicon for 1h but without impedance performance	94.4 ± 4.7

Each value reflects the average of three experiments, ± indicates standard deviation

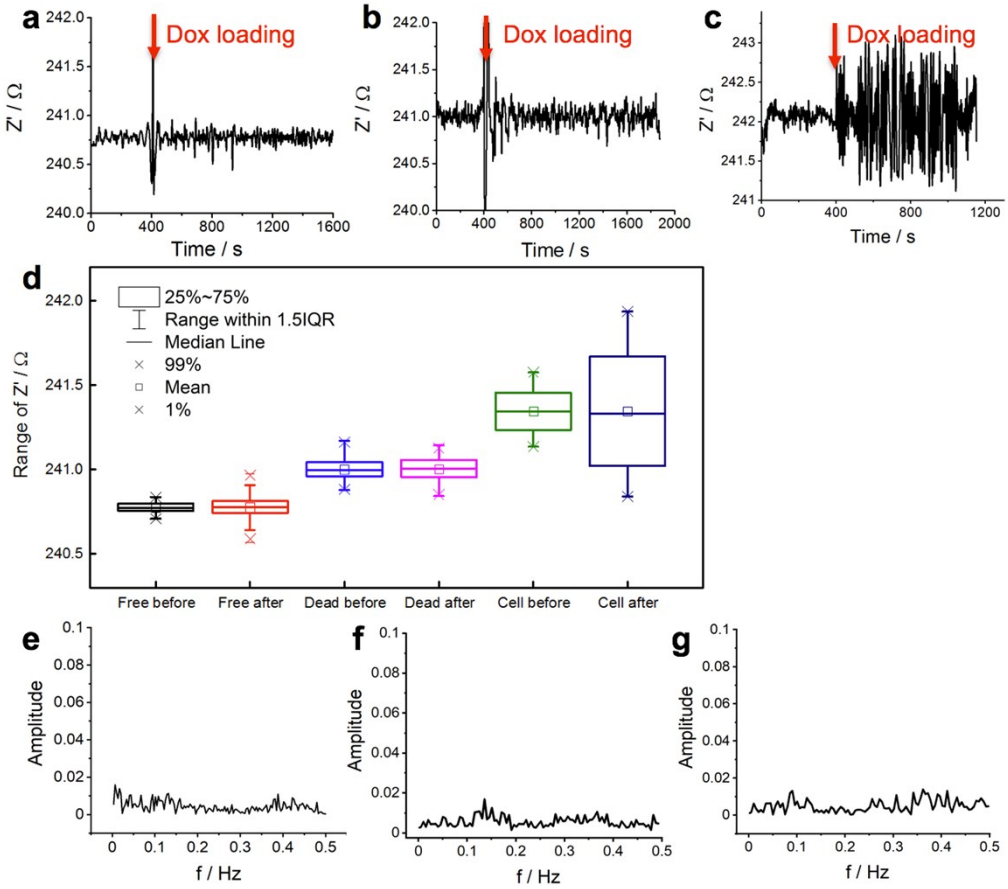

Fig. S3. Impedance recording before and after doxorubicin loading for (**a**) cell free region, (**b**) a dead cell as evidence from the cell membrane breaking down (but still attached on the surface), and (**c**) a living cell. (**d**) The box plots compare the impedance values ranges on the same silicon surface when performing impedance before and after loading doxorubicin on cell free region, a dead cell, and a living cell, respectively. FFT spectrum for (**e**) cell free region, (**f**) a dead cell after exposure to doxorubicin, and (**g**) a living cell before doxorubicin loading.

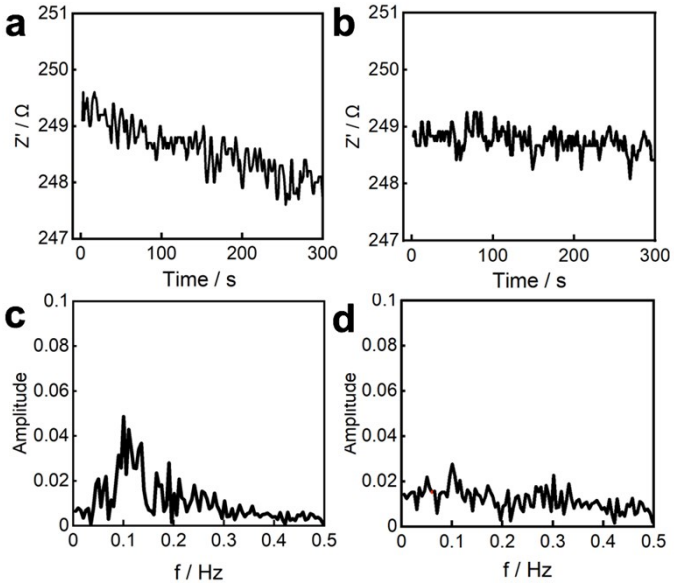

Fig. S4. More examples for the real part impedance curves (**a, b**) and the corresponding FFT spectrum (**c, d**) for different cells after loading doxorubicin.

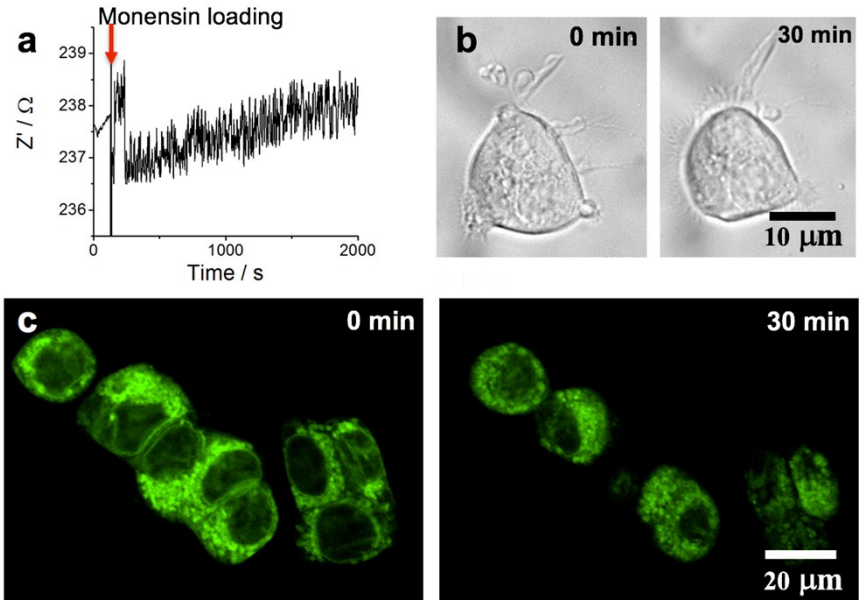

Fig. S5. Single cell impedance monitoring during 10 μM monensin treatment. (**a**) The bright field images for MCF-7 cells before (0 min) and after monensin treatment (30 min); (**b**) Impedance value recording for one single MCF-7 cell before and after loading 10 μM monensin; (**c**) 1 μM APG-2 AM was used to stain the intracellular K^+ and the fluorescence images clearly indicate the K^+ concentrations change inside of MCF-7 cells before (0 min) and after monensin treatment (30 min).

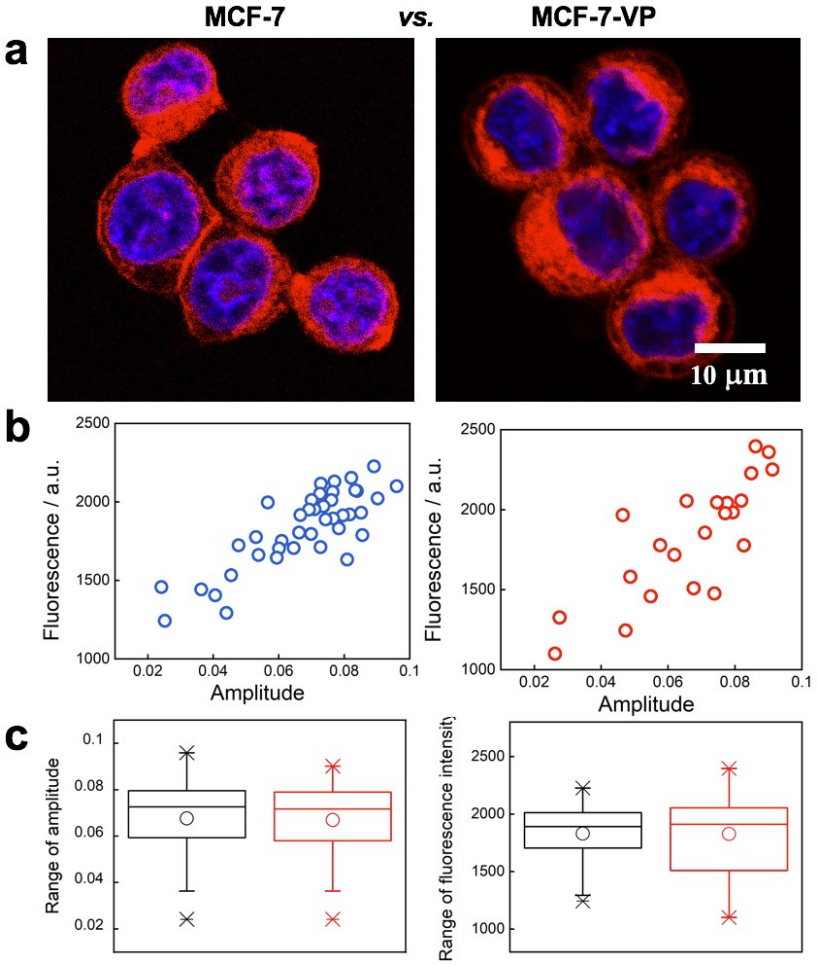

Fig. S6. (a) The fluorescent images for doxorubicin treated MCF-7 (left) and MCF-7-VP (right) after 30 min, blue for Hoechst 33342, red color reveals the accumulation of doxorubicin; (b) relationship between the fluorescence intensity of doxorubicin in MCF-7 cells and the amplitude obtained from impedance noise; (c) amplitude and fluorescence ranges for MCF-7 and MCF-7-VP.