Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Amorphous silicon on indium tin oxide: a transparent electrode for simultaneous light activated electrochemistry and optical microscopy

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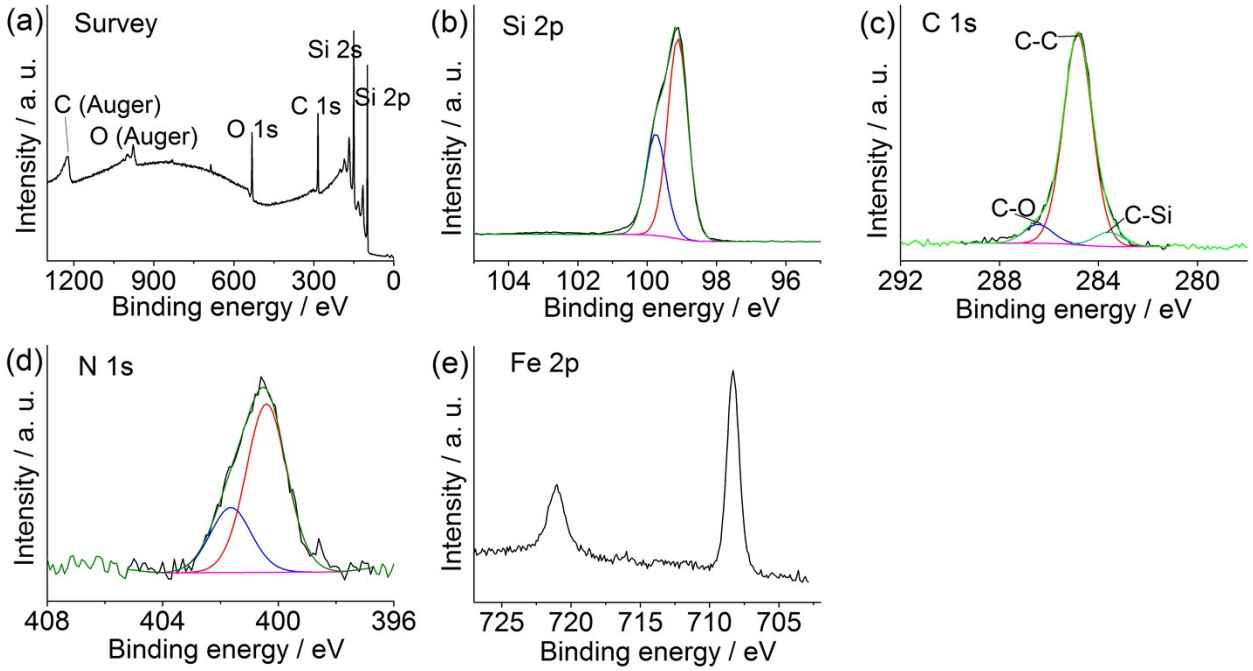

Figure S1. XPS spectra. (a) – (c), survey scan, Si 2p and C 1s narrow scans for the 1,8-nonadiyne hydrosilylated aSi-ITO. (d) – (e), N 1s and Fe 2p spectra of azidomethylferrocene modified aSi-ITO.

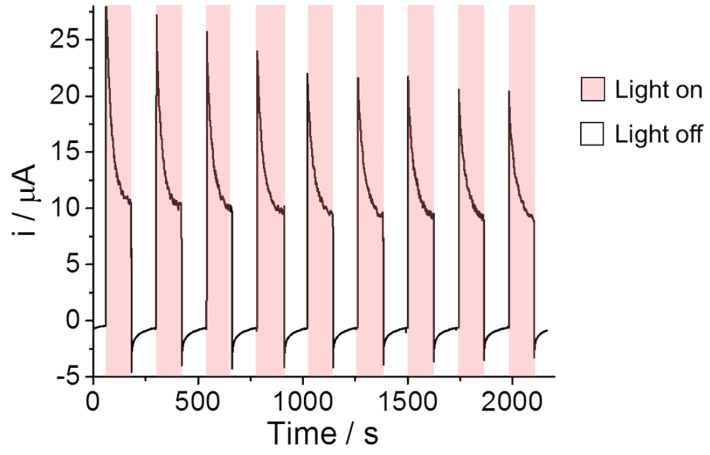

Figure S2. The amperometric trace of the ferrocene attached aSi-ITO electrode corresponding to the repeating light on-off cycles in the electrolyte containing 100 mM KNO_3 and 1 mM ferrocyanide. A constant potential 0 V vs reference electrode Ag | AgCl | 1 M KCl was applied over the whole experiment. The power of the light was 94.2 $mWcm^{-2}$. The ferrocyanide played a role to convert the oxidized ferricinium back to ferrocene so the ferrocene can be turned over continuously.

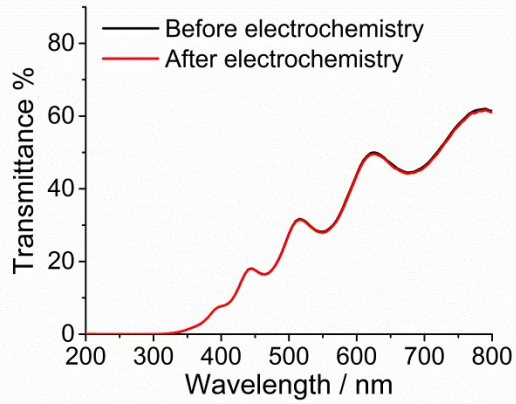

Figure S3. The transmittance % of the ferrocene functionalized aSi-ITO surface before and after the repeating light on-off electrochemical experiment in Figure S2.

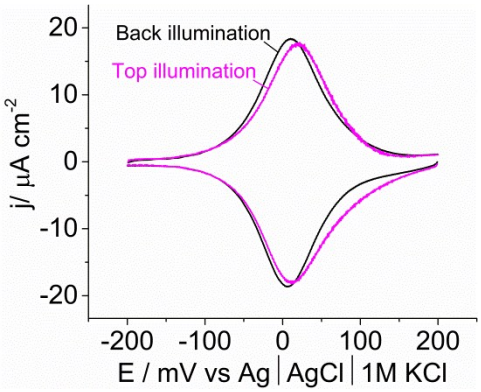

Figure S4. The cyclic voltammograms of the ferrocene attached aSi-ITO with backside and topside illumination. The scan rate was 100 mV/ s and the electrolyte was 1 M perchloric acid.

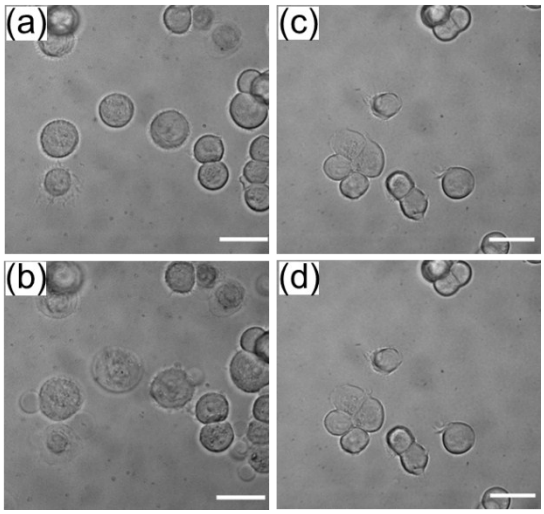

Figure S5. The bright field images of the MCF-7 cells (a) & (c) before and (b) & (d) after the ferrocene attached aSi-ITO surface was applied to - 0.7 V at 100 Hz for 750 s. (a) & (b) are cells from the area which was exposed to the light from the microscope objective, and (b) & (d) are cells from the area which has not been exposed to the light from the microscope during the application of the electric potential. Scale bars are 50 μm.

Experimental Methods

Chemicals and materials

ITO coated coverslip (18 x 18 mm, coverslip thickness # 1.5, 8 – 12 Ohms resistivity) was purchased from SPI Supplies. All chemicals, unless otherwise noted, were of analytical grade and use as received. Hydrofluoric acid (48 wt % in water) was semiconductive grade purchased from Riedel-Haen. Ethanol for substrate cleaning and propan-2-ol for reactions were redistilled prior to use. 1,8-Nonadiyne (Sigma-Aldrich) was redistilled over sodium borohydride (Sigma-Aldrich, ≥99%) at 60 °C under 25 – 30 Torr and stored in an argon atmosphere before use. Azidomethylferrocene was synthesized from a method reported earlier.[1] Milli-Q water (≥ 18 MΩ) for preparing reaction solution was collected from a Millipore water purification system. Copper sulfate pentahydrate was obtained from Chem Supply, Australia. Perchloric acid, sodium L-ascorbate and tetramethyl rhodamine B isothiocyanate (TRITC) conjugated phalloidin were from Sigma-Aldrich. Dulbecco's modified eagle medium (DMEM), Dulbecco's phosphate buffer saline (DPBS), Hoechst 33342 and the LIVE/DEAD® (calcein-AM / ethidium homodimer-1) Viability kit were from Life Technologies. HeLa and MCF-7 cell lines were purchased from CellBank Australia.

aSi-ITO preparation and their transparency measurement

Prior to aSi deposition, the ITO coated coverslips were sonicated in absolute ethanol and 0.5 M K_2CO_3 in methanol: Milli-Q water (3: 1, v/v) mixture for 10 min and 30 min, respectively. They were rinsed with a large amount of Milli-Q water, then with acetone and isopropanol, before dried. They were then subjected to plasma cleaning for 10 min. aSi film was deposited on an ITO coverslip using plasma-enhanced chemical vapour deposition (PECVD, Oxford Plasmalab 100). A small piece of Si wafer (about 3 mm x 3 mm) was placed at one corner of the ITO coverslip to block the aSi deposition at that site and this is the connection point to the external circuit in the electrochemical measurement. Deposition occurred at 300 °C, 25 sccm SiH_4 and 475 sccm He, 1500 mT process pressure and 20 W. Films with thickness 20 (±3) nm were prepared by controlling the deposition time. The thickness of the films was confirmed with ellipsometer (JA Woollam). The transmittance of the aSi-ITO samples at wavelength 200 – 800 nm were measured with a UV-Vis spectrophotometer. The samples were stabilized at a position that the incoming light source is normal to the middle of the sample.

Preparation of Ferrocene attached aSi-ITO

The aSi-ITO coverslip was treated with redistilled ethanol and subjected to plasma cleaning for 1 h to remove any organic contaminants on the surface. It was then dipped in 2.5% hydrofluoric acid (HF) solution for 90 s to remove the silicon oxide layer and generate a hydrogen-terminated

surface on the aSi. (Such a short etching process is negligible deleterious to the glass substrate. Careful that the uncoated ITO corner for connecting with electric circuit was not exposed to HF). After dried completely, the coverslip was immediately transferred to a Schlenk tube with 1, 8-nonadiyne which has been degassed through freeze-pump thaw cycles. A monolayer with alkyne group terminated was formed after the aSi-ITO sample was reacted with 1, 8-nonadiyne at 165°C for 3 h under Ar atmosphere. Unattached nonadiyne molecules were removed by rinsing the surface with copious amount of redistilled ethanol. Azidomethyl ferrocene was attached to alkyne-terminated aSi surface *via* the archetypal 'click' reaction. Azidomethyl ferrocene was firstly dissolved in isopropanol. It was mixed with sodium ascorbate aqueous solution and copper sulfate solution. The final concentration of azidomethyl ferrocene, sodium ascorbate and copper sulfate were 4 mg/mL, 1.66 mg/mL and 0.016 mg/mL, respectively. The alkyne-terminated aSi-ITO sample was reacted in the mixture solution at room temperature in the dark for 45 min. After the reaction, the surface was rinsed with copious amount of distilled ethanol and Milli Q water to remove unbound species.

Electrochemical measurement

Cyclic voltammetry (CV) of the aSi-ITO were carried out using BAS 100B electrochemical analyzer with a three-electrode cell. The azidomethyl ferrocene attached aSi-ITO were the working electrodes, while Ag|AgCl|KCl 1 M was the reference electrode and a platinum mesh was the counter electrode. A 94.2 mV lamp was used as the light source to illuminate the back side of the ITO substrate. $HClO_4$ (1 M) was the electrolyte solution for the ferrocene terminated sample. The aSi-ITO surface was subjected to voltammetry cycles at 100 mV/s under illumination until it showed a stable performance, which was called the 'conditioning step'. After that, the light source was off and the CV was collected again to reflect the electrochemical property of the aSi-ITO surface at dark. The repeating light on-off switching experiment was carried out using CHI potentiostat (660E). The ferrocene attached aSi-ITO was firstly subjected to the 'conditioning step' in 1 M $HClO_4$ then the electrolyte was changed to 1 mM ferrocyanide in 100 mM KNO_3 solution. A constant potential 0 V was applied continuously the whole experiment and the light was switched on and off manually every 120 s.

Fluorescence imaging of cells on aSi-ITO surface

HeLa cells were cultured in DMEM supplied with 10% fetal bovine serum in a 37 °C incubator with 5% CO_2. Trypsinized cell solution was diluted with DMEM and incubated on 20 nm aSi-ITO and normal glass coverslip (no. 1.5) in a 6-well plate for 6 h to allow cells to settle. They were then fixed with 4% paraformaldehyde in 1x PBS buffer for 30 min, followed by permeating with 0.1% Triton X-100 made in PBS for 15 min. The cells were blocked with 2% BSA in PBS solution for 30 min, before incubated with tetramethyl rhodamine B isothiocyanate (TRITC) conjugated phalloidin (for F-actin counterstain) and Hoescht 3342 stain in sequence for 40 min and 10 min, respectively. The samples were stored in PBS at 4˚C before imaging. Fluorescence images were obtained using an inverted confocal microscope (Zeiss LSM 780, 100 x objective, 1.4 numerical aperture).

Microscopy imaging cell lysis by light activated electrochemistry

About 1.7 x10^5 trypsinized MCF-7 cells in DMEM were added to a ferrocene functionalized aSi-ITO substrate which was assembled in a custom made electrochemical chamber and incubated

for 2 h. The cell medium was replaced with DMEM containing Calcein-AM and ethidium homodimer-1 stains. After further incubated at 37˚C for 20 min, the electrochemical cell was assembled using the aSi-ITO as the working electrode, a platinum mesh as the counter electrode and Ag│AgCl in 1 M KCl as the reference electrode on the stage of the fluorescence microscope. The cells were imaged using a fluorescence microscope (Olympus BX53). LIVE images were recorded with a FITC filter (excitation filter: 470/40, emission filter: 525/50 nm). DEAD images were recorded with a Cy3 filter (excitation filter: 545/50 nm, emission filter: 610/75 nm). After imaging, an electrical potential – 0.7 V, with amplitude 0.01 V and frequency 100 Hz was applied for 750 s using the CHI potentiostat, while the light from the objective of the microscope was still illuminating the same imaging area of surface. Fluorescence images were obtained again after 30 min to allow the ethidium homodimer-1 to penetrate to the cells damaged by the potential.

Reference

1. S. Ciampi, P. K. Eggers, G. Le Saux, M. James, J. B. Harper and J. J. Gooding, *Langmuir*, 2009, **25**, 2530-2539.